COMPLEX SYSTEMS APPROACH TO MODELING FOLATE METABOLISM: EXAMINING THE HOMOCYSTEINE REMETHYLATION PATHWAY

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Xuan-Mai Teresa Nguyen

January 2014

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Xuan-Mai Teresa Nguyen Ph.D.

Cornell University 2014

The overall objective of this research is to examine the joint effect of multiple variants in folate metabolism on CVD outcome. The intermediary outcome, homocysteine, will be investigated as the primary endpoint because the metabolic disruption characterized by elevated homocysteine levels is proposed to mediate the risk of CVD. Because epidemiologic studies are limited by small sample size, and thus reduced statistical power to examine genetic interactions and their combined effects on disease outcome, we utilize computer simulations to study five SNPs in four candidate genes that code for enzymes that are all linked through sequential metabolic steps in homocysteine remethylation. These enzymes are either directly involved in homocysteine remethylation or indirectly linked because they provide essential substrates required for the conversion of homocysteine to methionine by MTR. Using MTR as our focal point, we also considered gene-nutrient interactions among the five variants and varying levels of folate and vitamin B12 to account for the possible effects of nutritional status on disease risk. This approach led to the key finding that having double variants for all possible polymorphisms in a pathway does not necessarily equate to the most deleterious effects, and that only vitamin B12 had an effect on the homocysteine levels as a nutrient cofactor. Our simulations also illustrate how pathways have built-in regulatory mechanisms that researchers might not be able to account for when taking a single candidate gene approach to studying disease

outcome. We anticipate that our model will serve as an example of how simulations can help advance the growing idea that disease treatment can be personalized by examining an individual's unique genetic and nutritional profile.

BIOGRAPHICAL SKETCH

Xuan-Mai Nguyen was born to Canh Minh Nguyen and Ngoc-Nhung Viec in 1984 in the United States. She received her undergraduate degree in Applied Mathematics with an emphasis in Biostatistics at the University of California, Berkeley in 2004. Immediately following graduation, she began work on her M.S. and Ph.D. in Human Nutrition at the Division of Nutritional Sciences at Cornell University. For her Ph.D., she has minored in the fields of Biomedical Engineering and Genomics and has attended classes at the New England Complex Systems Institute in Cambridge, MA to further her training in modeling human metabolic pathways using a complex systems approach.

Dedicated to Bố, Mẹ, Nam and Bác Anh Teo–I'm finally done!

ACKNOWLEDGMENTS

This work would not have been possible without the understanding and amazing mentorship of Dr. Virginia Utermohlen, my Ph.D. advisor. She has provided the best combination of attentive listening, moral support and academic guidance throughout my graduate career. Her constant support has given me the opportunity to finally bridge my background in mathematics with my passion for understanding nutrition and disease. Dr. U's flexibility and faith in my abilities as a researcher and as an aspiring physician has been invaluable. I hope that I have not disappointed her!

I stumbled into your graduate BME class by accident, but I have never regretted taking your course or having you as my mentor, Dr. Peter Doerschuk. Your help during my journey to complete this dissertation has been incredible, and despite my rather scenic route through graduate school, I have finally arrived at my destination thanks to your encouragement and constant support.

Members of my graduate committee, Dr. Timothy O'Brien, Dr. David Lin and Dr. Zhenglong Gu who have been essential in shaping and inspiring the ideas presented in this dissertation.

My BSP family (John, Marco, Roger, Monica, Koy) and Maureen Lahiff at UC Berkeley for encouraging me to find my passion and to embark on this journey.

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CHAPTER 1

THEORETICAL MOTIVATION FOR COMPLEX SYSTEMS MODELING OF METABOLIC PATHWAYS

A. FOLATE METABOLISM AND CARDIOVASCULAR DISEASE

Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of adult morbidity and mortality worldwide, and is expected to remain as the primary cause of death through the year 2030 (1-2). The most common forms of CVD are multifactorial in origin, resulting from single or many genes working in combination with other genes (i.e. gene-gene interactions) and/or environmental factors to produce CVD risk (3). Monogenetic CVD can also arise due to a single genetic mutation in one of three genes involved in lipoprotein synthesis, but such incidences are rare (3-5). "Classical" risk factors for CVD are age, male gender, family history of premature CVD, cigarette smoking, hypertension, lipid abnormalities, diabetes mellitus, physical inactivity, poor diet, alcohol intake and obesity (6). Recently, other risk markers thought to play a causal role in CVD development have been identified, including biochemical indicators such as elevated plasma homocysteine (7).

Homocysteine: a biomarker for CVD

Homocysteine is a sulfur amino acid that forms as a result of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) demethylating the essential amino acid methionine (8). Total plasma homocysteine, which refers to all forms of homocysteine in plasma (i.e., free form or protein-bound (majority)), can be metabolized via two separate pathways: the irreversible transsulfuration pathway and the homocysteine remethylation pathway; the latter predominates under normal physiological conditions (9-10) (Figure 1.1).

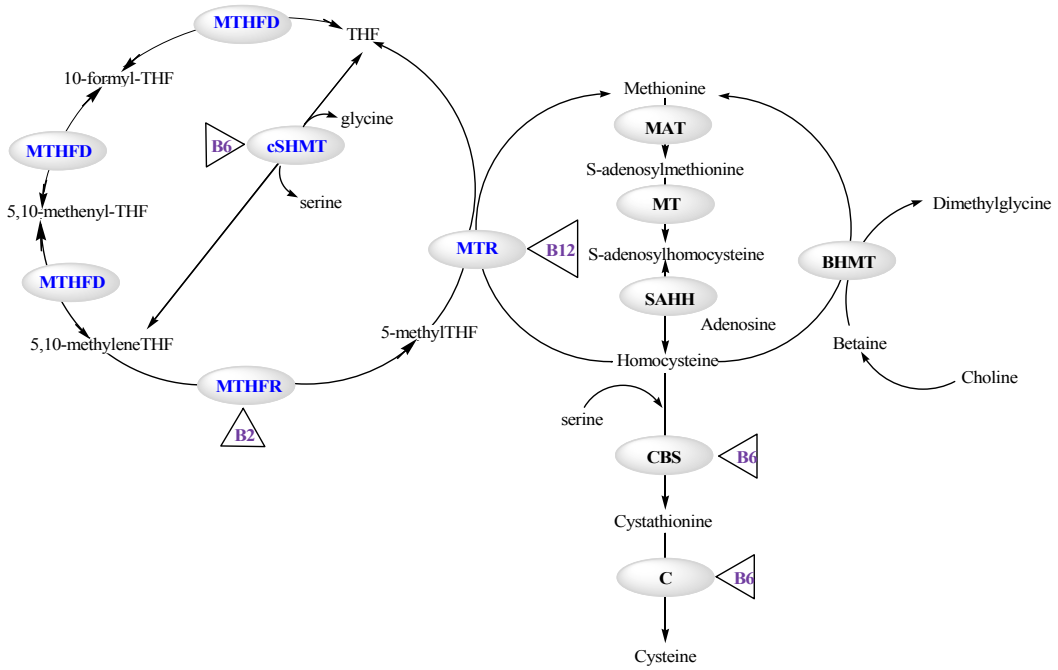


Figure 1.1: Overview of Homocysteine Metabolism

Major enzymes, nutrient cofactors and their roles in homocysteine remethylation: MAT = methionine adenosyltransferase; MT = methyltransferases; SAHH = S-adenosylhomocysteine hydrolase; BHMT = betaine-homocysteine methyltransferase; MTHFD = methylenetetrahydrofolate dehydrogenase; SHMT = serine hydroxymethyltransferase; CBS = cystathionine beta-synthase; C = gama-cystathionase; MTR = methionine synthase; MTHFR = methylenetetrahydrofolate reductase; THF = tetrahydrofolate; B_2, B_6, B_{12} = vitamins B_2, B_6, B_{12} , respectively.

Degradation of intracellular homocysteine to cysteine through the transsulfuration pathway is limited to liver and kidney cells and involves cystathionine beta-synthase (CBS) and γ-cystathionase, enzymes that both require pyridoxal 5'-phosphate (PLP; vitamin B6) as a cofactor while homocysteine remethylation occurs through two unique enzymatic reactions involving either methionine synthase (MTR) with 5-methyltetrahydrofolate (5-methylTHF) as the methyl donor or betaine-homocysteine methyltransferase (BHMT) with betaine as the donor of one-carbon units. Remethylation by BHMT is restricted to liver and kidney cells while remethylation by MTR can occur in every cell except red blood cells (8-10). The primary focus of this research is directed towards understanding the role of key factors involved in homocysteine remethylation through the MTR pathway where MTR is the major transmethylase (Figure 1.2).

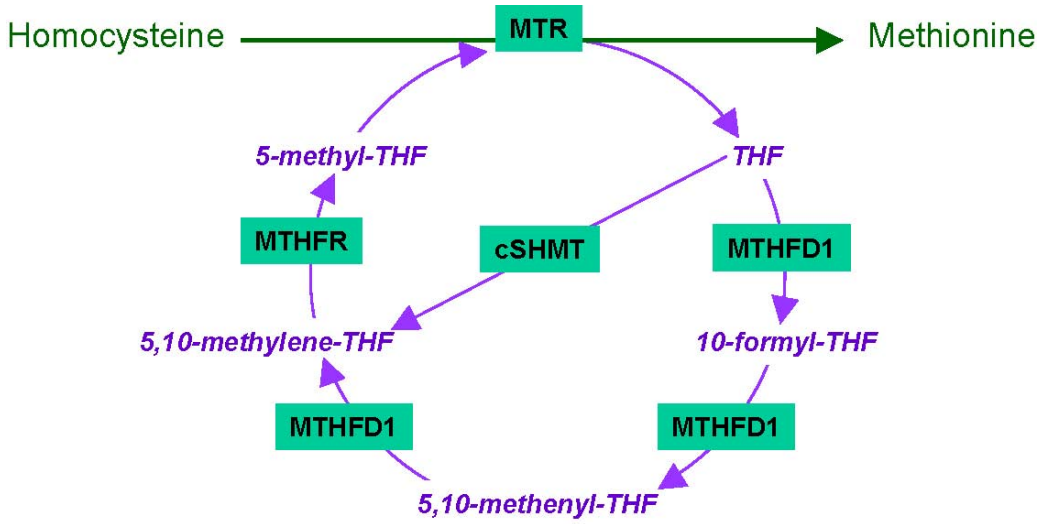


Figure 1.2: Homocysteine Remethylation through the MTR pathway
MTHFD = methylenetetrahydrofolate dehydrogenase; SHMT = serine hydroxymethyltransferase; MTR = methionine synthase; MTHFR = methylenetetrahydrofolate reductase; THF = tetrahydrofolate

Epidemiology of CVD

Studies taking the traditional reductionist approach have concluded that disruption of normal enzymatic function within the homocysteine remethylation pathway through MTR can result in an accumulation of homocysteine, leading to increased CVD risk. Meta-analyses of epidemiologic studies have found that total plasma homocysteine is an independent predictor of CVD (11-12). Results from a meta-analysis conducted in 2002 of prospective studies estimated that a decrease in total plasma homocysteine of 3umol/L was associated with a 16% decrease in heart disease (12). Similarly, a meta-analysis by the Homocysteine Studies Collaboration that included studies published between January 1966 and January 1999 reported that a 25% lower than usual homocysteine level (~3 umol/L; 0.41mg/L) was associated with an 11% lower risk of ischemic heart disease (OR= 0.89; 95% CI: 0.83-0.96) (11). The mechanisms by which elevated homocysteine affects CVD risk are not completely specified, but it has been hypothesized that elevated homocysteine concentrations cause: (a) endothelial dysfunction by impairing nitric oxide synthesis (b) platelet activation, (c) a pro-inflammatory response by inducing production of tumor necrosis factor-alpha and (d) accelerated oxidation of low-density lipoproteins (LDL), and thus, atherosclerosis (6,7,10-11, 13-24). The proposed mechanisms by which homocysteine increases CVD risk support epidemiologic findings that report an association between increased blood homocysteine concentrations among individuals with vascular disease, where elevated homocysteine levels precede CVD onset (25-29).

Genetic and Nutritional Determinants of Homocysteine Remethylation and CVD Risk

The homocysteine remethylation pathway via MTR is embedded within the larger one-carbon folate metabolic pathway (Figure 1.3). Studies have demonstrated an association between increased blood homocysteine concentrations and changes in individual states of oxidation as well as the addition or removal of one-carbon groups

like folate (30). MTR, a vitamin B12-dependent enzyme, catalyzes the methyl group transfer from 5-methylTHF to homocysteine, resulting in methionine and THF (31). The proper functioning of this enzyme is critical for ensuring that homocysteine levels do not reach toxic levels. It is, thus, biologically plausible that inadequate levels of vitamin B12 or disruption in MTR function can affect MTR activity level, which in turn can lead to hyperhomocysteinemia as well as homocysteinuria (32).

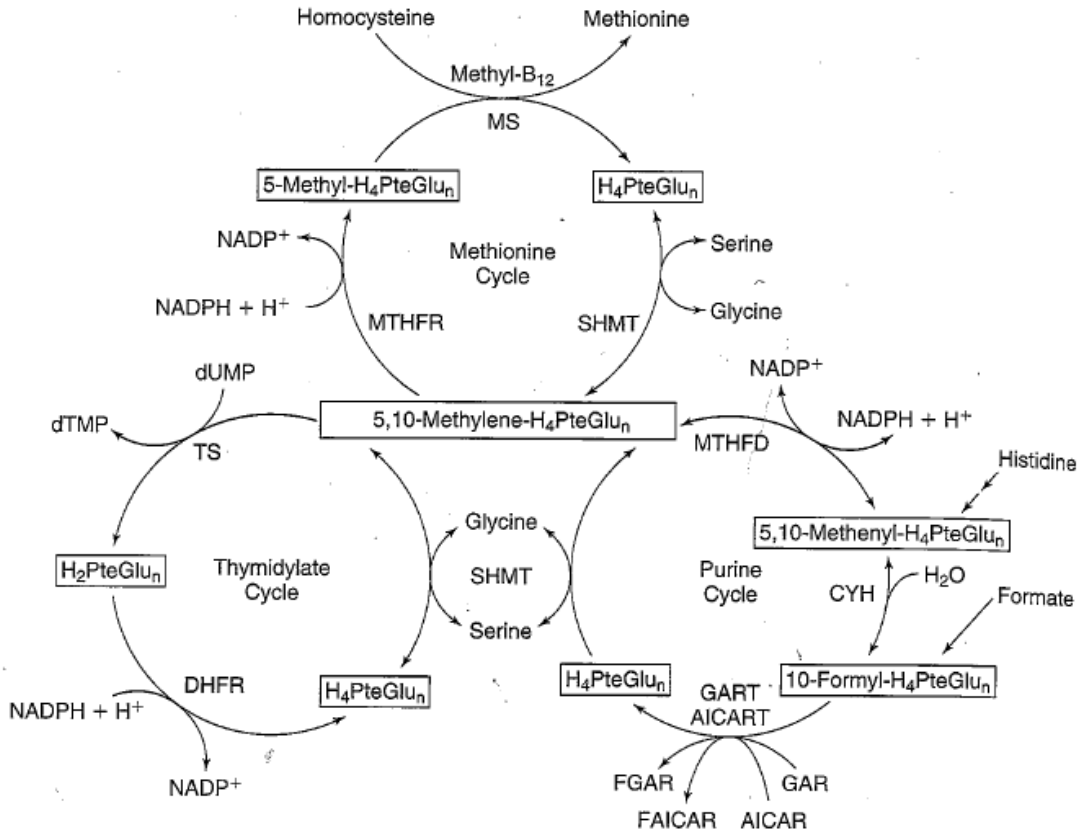


Figure 1.3: Global Folate Metabolic Network, adapted from Stipanuk et al. (33) These are the major folate-dependent one carbon metabolic pathways in the cytoplasm of the cell. AICAR: 5-amino-4-imidazolecarboxamid ribonucleotide; AICART: AICAR formyltransferase; CYH: methenyltetrahydrofolate cyclohydrolase; DHFR: dihydrofolate reductase; dTMP: 2-deoxythymidine 5'-monophosphate; dUMP: 2-deoxyuridine 5'-monophosphate; GAR: glycinamide ribonucleotide; GART: GAR formyltransferase; MS (MTR): methionine synthase; MTHFD: methylenetetrahydrofolate dehydrogenase; MTHFR methylenetetrahydrofolate reductase; (c)SHMT: cytoplasmic serine hydroxymethyltransferase; TS: thymidylate synthase.

A common single nucleotide polymorphism (SNP) in *MTR* consists of an A-to-G nucleotide substitution at base pair 2756 (rs1805087), leading to an amino acid change from aspartic acid to glycine at codon 919 (34). Functional consequences of this mutation have not been clearly established, and the influence of the *MTR* 2756 A→G polymorphism on total plasma homocysteine levels is still a matter of debate. Some studies suggest that individuals with *MTR* 2756 *AA* genotype have lower homocysteine concentrations (compared to those with *MTR* 2756 *GG* genotype), implying an increased level of enzymatic activity in the presence of the variant genotype (35-38). Other studies have reported either no functional differences (39-42) or an increase in homocysteine concentration as a result of the *MTR* 2756 A→G polymorphism (37, 43). In laboratory studies, complete loss of MTR activity in mice has been linked to early embryonic lethality (44). Similarly, severe clinical consequences have been observed in humans lacking MTR activity (45).

With regard to nutrient cofactors, traditional gene association studies hypothesize that the *MTR* 2756 A→G polymorphism may decrease the ability of vitamin B12 to bind to its receptor site on MTR, in part because the SNP is in close proximity to the vitamin B12 binding domain of the protein. The role of vitamin B12 as a cofactor is critical for proper MTR function because it serves as an intermediary in methyl transfers catalyzed by MTR (46). Thus, the SNP is hypothesized to increase the risk of CVD (less remethylation of homocysteine) as well as increase cellular B12 levels (less binding of B12 to enzyme). With respect to plasma vitamin B12 levels, prior work has shown that reduced levels of plasma vitamin B12 are associated with higher homocysteine levels and thus, greater CVD risk (47). Typically about 20% of total circulating vitamin B12 is carried by transcobalamin 2, which is responsible for transporting vitamin B12 in the blood to the cell, and a reduction in total circulating vitamin B12 levels will proportionally reduce the availability of vitamin B12 to bind

to transcobalamin 2, causing a deficit in vitamin B12 availability for MTR to use as a cofactor to remethylate homocysteine. Studies examining the association of *MTR* 2756 A→G with circulating vitamin B12 levels have produced mixed results: some report no association between *MTR* 2756 A→G genotype and blood vitamin B12 levels (37, 41, 48-49) and others report a decrease of ~30% in serum vitamin B12 levels by *MTR* 2756 A→G genotype (*MTR* 2756 *GG* vs. *MTR* 2756 *AA/AG*) (40, 50). To the best of our knowledge, whether the effect of the polymorphism on cardiovascular disease risk varies by nutritional status of vitamin B12 has not been studied to date. However, results from animal studies support the idea that vitamin B12 concentration plays a critical role in stabilizing MTR activity as induced B12-deficient rats had severely reduced MTR activity levels compared to the enzyme activity levels of rats in a control group who were not B12-deficient (51).

Homocysteine remethylation by MTR also requires 5-methylTHF as a substrate, which is provided by a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR). This key enzyme has been extensively studied in relation to CVD pathogenesis because MTHFR is responsible for the reduction of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) to 5-methylTHF, the active folate derivative required for homocysteine remethylation. Insufficient levels of this major form of folate in the human body can result in hyperhomocysteinemia (52-53). A single base, non-synonymous substitution of C to T at nucleotide 677 (rs1801133), leading to an alanine to valine amino acid substitution at codon 222, has been identified where a missense mutation in the region encoding the N-terminal catalytic domain results in a thermolabile variant with a 50% reduction in enzymatic activity (54-55). This variant has been consistently associated with mildly elevated plasma homocysteine levels (52, 56) and has been found to be more prevalent among individuals with CVD than those without (55, 57). Given that *MTHFR* 677 C→T is

the most widely studied polymorphism in folate metabolism, we seek to understand how the combined effects of other polymorphisms affecting enzymes in folate metabolism influence the relation of *MTHFR* 677 C→T with elevated homocysteine levels.

The thermolabile *MTHFR* 677 C→T polymorphism accounts for mild hyperhomocysteinemia in only about 25% of vascular disease patients (52), suggesting that additional mutations in the *MTHFR* gene, or in related genes, may contribute to elevated homocysteine levels. One such polymorphism found in the C-terminal regulatory domain of MTHFR is *MTHFR* 1298 A→C (rs1801131), which leads to a glutamine to alanine amino acid substitution (54,58). Studies taking a single candidate gene approach have found no relation between the *MTHFR* 1298 A→C polymorphism, homocysteine levels and risk for CVD (59-62). However, results from observational studies looking at the combined effects of *MTHFR* 1298 A→C and *MTHFR* 677 C→T have produced mixed results: several studies reported no additional explanatory power in *MTHFR* 1298 A→C for predicting coronary disease outcome once the *MTHFR* 677 C→T SNP was considered (59-62), but other reports suggest that combined heterozygosity for *MTHFR* 677 C→T and *MTHFR* 1298 A→C mutations is associated with hyperhomocysteinemia (58). More specifically, van der Put et al. examined the effects of *MTHFR* 1298 A→C on homocysteine levels and CVD risk and demonstrated an interactive effect: individuals heterozygous for both *MTHFR* 677 C→T and *MTHFR* 1298 A→C had reduced MTHFR specific activity (ANOVA $p<0.0001$), higher homocysteine levels and decreased plasma folate levels (ANOVA $p<0.03$) (58). The effect of combined heterozygosity was also shown by Lievers et al. and Weisberg et al. who noted that individuals with *MTHFR* 677 *CT*/1298 *AC* genotype had slightly higher homocysteine levels than those with the *MTHFR* 677 *CT* /1298 *AA* genotype (54, 63-64). When folate status was additionally

accounted for, Weisberg et al. found that the relation between *MTHFR* 1298 A→C genotype and homocysteine levels was stronger among those with folate levels below the median than those with levels above the median, though the association was not statistically significant (data not shown in publication) (54). Finally, an *in vitro* study directly assessing the effect of *MTHFR* 1298 A→C on enzyme activity and thermolability concluded that the *MTHFR* 677 C→T variant was more deleterious than the alanine variant of *MTHFR* 1298 A→C (54) , suggesting that the role of *MTHFR* 1298 A→C on MTHFR enzyme activity is minor compared to that of *MTHFR* 677 C→T . Overall, the evidence to date is suggests that the effect of *MTHFR* 1298 A→C is not observed to have an effect on clinical outcomes unless there is a reduced folate status or there is also a variant allele at the 677 nucleotide (*MTHFR* 677 C→T). In our current study, a mathematical model including both *MTHFR* SNPs will allow for a complete examination of the *MTHFR* genotype combinations as they relate to homocysteine levels.

In addition to genetic factors, MTHFR activity is also influenced by levels of folate. Epidemiology studies have found that in populations with low folate status the associations among the *MTHFR* 677 C→T polymorphism, CVD risk, and elevated homocysteine are more pronounced (53, 65). However, when folate levels are higher, the net effect of the *MTHFR* 677 C→T polymorphism on homocysteine levels is attenuated (65), highlighting an important gene-nutrient interaction between folate and *MTHFR* 677 C→T.

The rate of conversion of 5,10-methyleneTHF to 5-methylTHF, which is needed for homocysteine remethylation by MTR, is also dependent on the availability of 5,10-methyleneTHF. Cytosolic serine hydroxymethyltransferase (cSHMT) and methylenetetrahydrofolate dehydrogenase (MTHFD1) are both enzymes that catalyze reactions producing 5,10-methyleneTHF. Metabolic disruptions caused by changes in

activity in these enzymes make cSHMT and MTHFD1 important producers of a substrate that may ultimately influence homocysteine remethylation. cSHMT is a key enzyme responsible for regulating and maintaining the homeostasis of the intracellular one-carbon pool. It supplies one-carbon units for thymidylate biosynthesis, sequesters 5-methylTHF causing reduced S-adenosylmethionine synthesis, and catalyzes glycine-dependent serine synthesis which depletes 5,10-methyleneTHF for homocysteine remethylation (66).

A SNP in *cSHMT*, *cSHMT* 1420 C→T, was identified by Stover et al., and causes an amino acid change from leucine to phenylalanine (67-68). Recent studies find that *cSHMT* 1420 C→T polymorphism (rs1979277) is located close to the sumoylation site on the cSHMT protein, which affects nuclear localization of the protein (69). Epidemiologic studies have implicated this polymorphism in a variety of diseases including leukemia (70) and lymphoma (71-72), esophageal squamous cell carcinoma and gastric cardia adenocarcinoma (73) and neural tube defects (74). *cSHMT* 1420 C→T has also been investigated in relation to CVD outcome: findings show that the effect of *MTHFR* 677 C→T on CVD risk is strongly influenced by *cSHMT* 1420 C→T genotype. Specifically, among men with *cSHMT* 1420 *TT* genotype, the risk of CVD for *MTHFR* 677 *CT* and *TT* genotypes was 3.6 (95% CI: 1.7,7-8) and 10.6 (95% CI: 2.5, 46.0), respectively (compared to *MTHFR* 677 *CC* genotype). Among men with *cSHMT* 1420 *CC/CT* genotype the risk for CVD among *MTHFR* 677 *CT* and *TT* genotypes (compared to *MTHFR* 677 *CC*) was 1.0 (95% CI: 0.8, 1.2) and 1.3 (95% CI: 0.9, 1.8), respectively (75). Whether this increase risk is a result of elevated homocysteine levels was not determined.

This evidence suggests that the metabolic disruptions resulting from *cSHMT* 1420 C→T have the potential to play a role in health outcomes, particularly in CVD pathogenesis, through gene-gene interactions. Since the study by Lim et al. (75) found

an effect resulting from a pair-wise interaction between polymorphisms in *cSHMT* and *MTHFR* on CVD risk, it is plausible that these polymorphisms may also interact with SNPs in *MTR* or *MTHFD1* from the epidemiology perspective. Again, the relationship of any clinical consequence to hyperhomocysteinemia remains unknown.

MTHFD1 is a tri-functional enzyme that catalyzes three sequential reactions responsible for the interconversion of 5,10-methyleneTHF and THF. A polymorphism in the coding region, *MTHFD1* 1958 G→A (R653Q; rs2236225) has been implicated as a cause of neural tube defects (76-77), bipolar disorder and schizophrenia (78) and is associated with the risk for migraine and gastric cancer through an interaction with the *MTHFR* 677 C→T genotype (79-80). Because MTHFD1 provides the essential substrate for MTHFR, mutations in the *MTHFD1* gene that affect enzyme activity are hypothesized to influence levels of homocysteine, and consequently, CVD risk. Only one study has examined the effects of *MTHFD1* 1958 G→A and *MTHFR* 677 C→T on CVD risk (Raiszadeh, personal communication). Findings suggest that *MTHFD1 GA/AA* (vs. *MTHFD1 GG*) had a statistically non-significant protective association with CVD risk (OR 0.8; 95% CI 0.6, 1.1), and this association was similar across folate subgroups. A gene-gene interaction was observed whereby an increased risk of CVD was found for the *MTHFR* 677 *TT* genotype (vs. *MTHFR* 677 *CC*), but only among men with *MTHFD1 GA/AA* genotype (OR 1.6, 95% CI 1.1, 2.4). Similarly, the *MTHFR* 677 *CT* genotype (vs. *MTHFR* 677 *CC*) increased CVD risk among men with *MTHFD1 GA/AA* genotype (OR 1.2; 95% CI 0.9, 1.7), but had little or no effect in men with the *MTHFD1 GG* genotype (OR 0.8; 95% CI 0.5, 1.3) (Raiszadeh, personal communication). No other study to date has examined the role of multiple polymorphisms that include *MTHFD1* 1958 G→A on CVD risk by examining the changes in homocysteine levels.

Taking a new approach to understanding cardiovascular disease, the research presented in the following chapters examines the interactive effect of 5 SNPs in genes coding for enzymes in folate metabolism on CVD outcome by looking at changes in homocysteine steady state concentrations as a proxy (Table 1.1). To the best of our knowledge, no published study to date has considered more than one gene-gene interaction in genes within the homocysteine remethylation pathway in relation to CVD outcome. Moreover, the majority of studies to date have taken an epidemiologic approach to understanding folate metabolism and its relation to homocysteine and cardiovascular disease. While epidemiologic gene association studies help identify correlations between single genetic variables and disease outcomes, such an isolated approach rarely leads to the formulation of relations that can remain true when entities of a biological system are examined as a whole with respect to disease outcome. Thus, to understand how genetic polymorphisms affect enzymes in homocysteine remethylation as a whole, we propose a new method of analyzing biological systems using complex systems analysis.

Table 1.1: Five Gene and SNP Information

Gene	SNP	Amino Acid Change	MAF[1]
cSHMT	1420 C→T	Leu474Phe	HapMap CEU: 0.333
17p11.2[2]	rs1979277		NAS: 0.310
MTHFD1	1958 G→A	Arg653Gln	HapMap CEU: 0.458
14q24	rs2236225		NAS: 0.465
MTHFR	677 C→T	Ala222Val	HapMap CEU: 0.242
1p36.3	rs1801133		NAS: 0.363
	1298 A→C	Glu429Ala	HapMap CEU: 0.358
	rs1801131		NAS: 0.306
MTR	2756 A→G	Asp919Gly	HapMap CEU: 0.167
1q43	rs1805087		NAS: 0.181

[1] Minor Allele Frequency for each of the 5 SNPs assayed in the Normative Aging Study population (NAS), which consists of white males with European ancestry, compared to the CEU reference population, which refers to the subgroup of people living in Utah with ancestry from northern and western Europe in the International Haplotype Mapping (HapMap) Project (81)
[2]Chromosome location

B. THE ROLE OF COMPLEX SYSTEMS METHODS IN METABOLIC MODELING

Systems Biology and Complex Systems Approach

The discipline of systems biology focuses on addressing the great intellectual and technical challenges associated with translating genome sequence into a comprehensive understanding of how organisms are built and run. It is rooted in the idea organisms are more than the sum of their parts, and the behavior of their physiological processes cannot be understood by simply knowing how the parts work in isolation. A systems level characterization of a biological process addresses three fundamental questions: (i.) what are the parts of the system? (i.e., the genes and the enzymes and proteins that they code); (ii.) how do the parts work? (iii.) how do the parts work together to complete a task? (82).

A system is a relation of items. Items within a system take on any range of identities including physical objects, indicators, variables or even symbols. For example, a genome is a set of genes and a metabolic pathway can be described by rate equations. In mathematical set theory, a system can be defined as the set of all ways in which an item A_i can be found in the system, where * denotes that all items are seen simultaneously.

$$S \subset A_1 * A_2 * \ldots\ldots * A_i \ldots * \ldots . A_n$$

Here, an element of S represents a multiple of the elements in each of the items, s = (i_1, …..i_n). Items are mutually interdependent, therefore, they constitute a relation.

A complex system is therefore defined by the following: (i.) the global system has a distinct behavior and (ii.) the subsystems within the global system preserve their own identities and inherent properties. Symbolically, a complex system can be represented as:

$$S \subset S_1 * S_2 * \ldots\ldots * S_i \ldots * \ldots . S_n$$

In describing a complex system, "distinct" behaviors imply that the behavior of the complex overall system is described in terms of items (i.e., subsystems) that are different than the items used to describe the subsystem components. For example, Figure 1.3 illustrates the folate metabolic network, an entity that can be considered as a global system. Here, there are three subsystems that describe the global system: the methionine cycle, thymidylate cycle and the purine cycle. Furthermore, each subsystem is characterized by items such as enzyme kinetics, substrate concentration, product concentration and nutrient cofactor levels. Figure 1.1 illustrates the items for describing the methionine cycle subsystem. The items used to describe the subsystems that form a complex system have their own boundaries and existence, yet their behavior and function is conditioned by their presence within the overall global, complex system. Specifically, the methionine cycle, thymidylate cycle and purine cycle are all acknowledged as such, but their functioning and existence is conditioned as being part of the total system (i.e., folate metabolism).

Principles of organized complexity

A major challenge that has yet to be fully addressed in systems biology is the development of principles of organized complexity in biological systems. These principles, in theory, should provide a starting framework to uncover the understanding of systems from observation and data. Moreover, these principles should indicate the functions of biological systems and/or their components. They provide the "architecture" of the model and may not necessarily be numerically based.

Levels of complexity and the autonomy of levels

The idea of multilevel-ness is an essential principle of organized complexity. A biological example of multilevel-ness can be seen in the study of CVD. CVD is a complex event that can be studied from a variety of perspectives. Genetic variables, small molecule and protein interactions as well as epidemiological trends are viable

methods of studying CVD. Each method provides a subset of information explaining some aspect of CVD. How all of the subsets can be combined or studied concurrently to provide an understanding at the global level lies at the heart of systems biology from a complex systems approach. The concern in systems biology and complex systems is, therefore, to understand how the functioning of a higher level (i.e., the global level) is related to the functioning on a lower level (Figure 1.4).

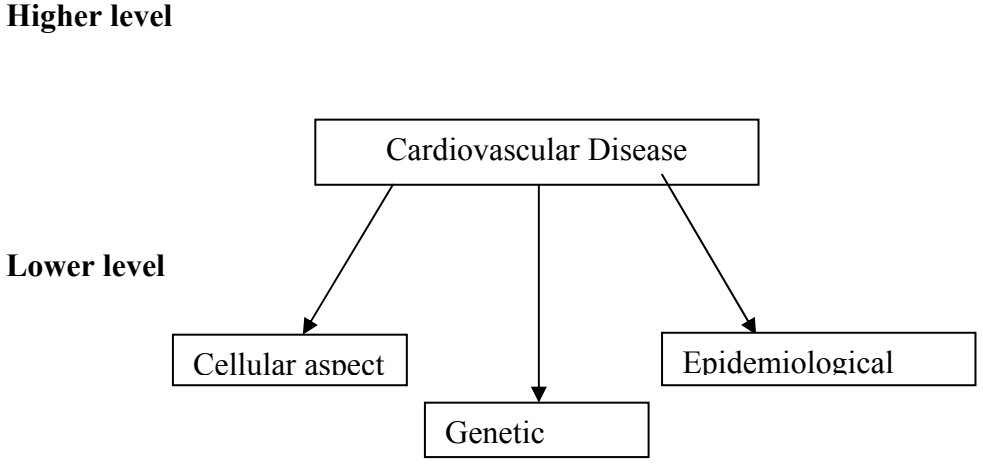


Figure 1.4: Multilevel-ness in the study of cardiovascular disease

The study of complex systems is "a new field of science studying how parts of a system give rise to the collective behaviors of the system, ***and*** how the system interacts with it environment…It focuses on certain questions about parts, wholes and relationships…[It] is about understanding indirect effects" **(**83). Complex systems, therefore, is integrative and seeks to understand and predict the behavior or "emergent" properties of complex, multicomponent biological processes. Here, emergent is defined as the distinct behavior on a higher level that is solely due to the way the subsystems on the lower functioning level interact.

As Stuart Kauffmann suggested, there are domains of autonomy on the lower level of subsystems that do not affect the behavior of the system at the higher level. The domain of autonomy is a range of changes on the lower level and the corresponding range of normal behavior on the higher level such that the two levels do not interact; the changes on the functional level are treated as "background noise" on the higher level (84). This brings up the need to clarify the idea of interaction and interdependence among levels and subsystems within a complex system, namely, that even though levels are interdependent in many ways, they can be viewed as non-interacting within domains of autonomy. In the case of folate metabolism, all three subsystems (i.e., methionine, thymidylate and purine cycles) are all interdependent on each other via 5,10-methylenetetrahydrofolate. While enzymes and cofactors in each cycle will perturb reaction rates and equilibrium within their respective cycles, whether or not these perturbations of the "subsystems" are manifested in the global properties that drive folate metabolism is best measured using a complex systems approach.

Interactions and Links

When a system is "complex," its subsystems and items defining each subsystem are interdependent and a quantifiable description of each item of varying levels requires

algorithmic complexity. Algorithmic complexity, is defined as the quantity of items needed to describe the system (or subsystem) and the number of clauses to define the relationships/interactions among the items is large (i.e., it cannot be described with a single-worded answer or using a single sentence). The descriptions for items at each level vary. At the higher level, the purpose is to **coordinate** the subsystems to perform work. Thus, the function of interactions at the higher level should consist of providing guidance or motivation for the subsystems to act so as to advance the overall system's objective. It is necessary to organize the functioning of subsystems so that the overall system, as a whole, functions properly (84). In the case of folate metabolism, this means that at the higher level of complexity, the folate metabolic system, as represented in Figure 1.5 as $CP(\Delta_p)$, is concerned with ensuring that thymidylate, methionine and purine cycles ($S_i[\Delta_i(\beta)]$, where $\Delta_1 \ldots \Delta_n$ represent regulation objectives) are all operating such that folate metabolic system will continue to function. Coordination theory (85) suggests that coordination can affect the first level of subsystems (i.e., the three cycles) by an input β to modify their functioning. These three subsystems are, in turn, responsible for their own functions at a lower level (i.e., regulating their enzymes/items) and are modified by coordination at the higher level. In other words, according to coordination theory, let Δ represent the functioning of the system as a whole and satΔ indicate that the functioning of the overall system is satisfactory. The purpose of the coordination task, Δ_p, is to influence all $\Delta_i(\beta)$ so that Δ is achieved while the first level systems (i.e., the subsystems) perform their own, first (lower) level functions where:

$$[sat\Delta_1(\beta) \cap \ldots sat\Delta_i(\beta) \cap \ldots sat\Delta_n(\beta)] \cap sat\Delta_p \Rightarrow sat\Delta.$$

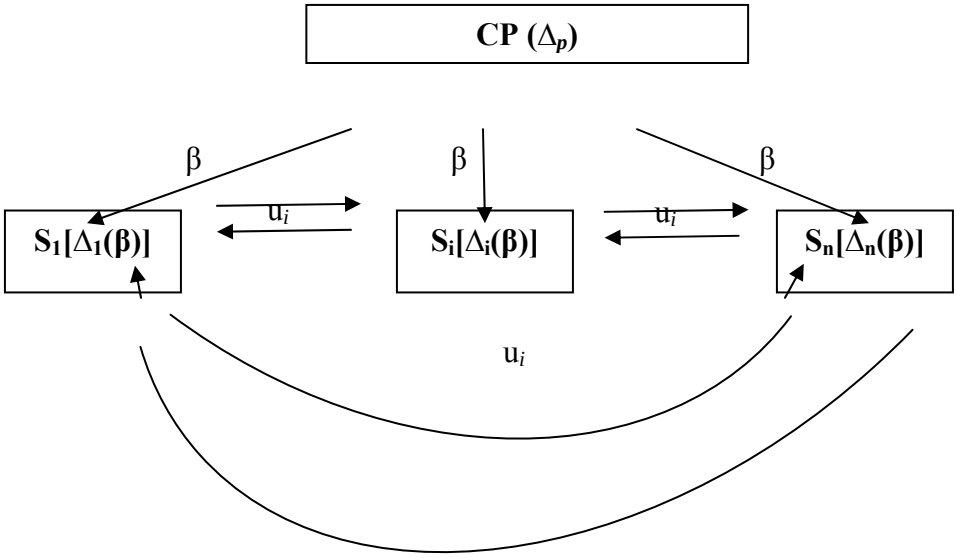


Figure 1.5: Coordination by a coordination process
S_i is the first functional level subsystem; CP is the coordination process; Δ_p is the coordination task; Δ_i is the first level regulatory function; u_i is the interaction between subsystems; β is the coordination input

Coordination is also necessary if perturbations are made to the overall system. Such perturbations, denoted as x, such as genetic mutations, can affect a subsystem's ability to produce the correct amount and type (i.e., an enzyme) of information for the ideal, proper functioning of the overall system. When such a discrepancy arises, coordination is needed to bring equilibrium back to the coordination process and the system. Specifically, the coordination task, Δ_p, then has a purpose to find β such that the following holds true:

$$[sat\Delta_1(x,\beta) \cap \ldots sat\Delta_i(x,\beta) \cap \ldots sat\Delta_n(x,\beta)] \cap sat\Delta_p \Rightarrow sat\Delta.$$

Sometimes, these perturbations cannot be corrected and a system's equilibrium cannot be achieved. In this case, it is thought that over time, the system will adapt to reach a new state of equilibrium (85). More specifically, as with many biological phenomena, slight perturbations (i.e., single nucleotide polymorphisms) are often not fatal because biological systems are robust and resilient to change. This suggests that even with mutations, a biological system can tolerate deviations and imbalances and adapt within certain bounds to continue proper function at a reduced level of optimization. It is of interest to determine what these bounds are and how a system adapts. Using complex systems analysis, the bounds of a given metabolic pathways and perturbations within the pathway could theoretically be examined.

Gene-Disease Modeling: A Case for the Complex Systems Approach

Developing a systems level understanding of a physiological process requires identification of the genes and the proteins that they encode (i.e., the "parts"). The field of functional genomics is one that has developed and utilized large-scale and high-throughput methodologies to define and analyze gene function by integrating data obtained from multiple large-scale datasets (86-91). Traditional reductionism has also given us a deeper understanding of the parts involved in the organizational structure of biological processes. It has elucidated the importance of cellular

components and regulatory processes of specific genes, proteins and metabolites. In a sense, reductionism has established the foundation upon which the behavior of complex physiological processes can be studied as a whole.

As the writer Alvin Toffler once said, "One of the most highly developed skills in contemporary Western civilization is dissection: the split-up of problems into their smallest possible components. We are good at it. So good, we often forget to put the pieces back together again" (92). In the world of gene association studies, we see exactly what Toffler described, namely, we know a lot about the association of a single gene with a single disease outcome, but we fail to acknowledge the interdependency among different genes that code for enzymes and proteins that interact with one another (93).

Current strategies to understand the role of genetic variation in various clinical phenotypes illustrates the growing need for a complex systems approach to studying the relationship between genetic information and human disease. Original methodologies designed to discover links between genetic information and human disease have traditionally focused on the evaluation of candidate genes identified by classical reductionist techniques in the laboratory (94). This methodology relies on existing knowledge of the genes to determine the properties of important cell systems presumed to be disturbed in a disease state. Additionally, candidate gene studies have been characterized by the examination of genetic variation in a single gene, which ignores the interaction between related genes coding for proteins in the same pathway. Because investigation tends to be limited to genes of known function that have been linked to the pathophysiology of a disease in question, candidate gene studies can—and have- produced conflicting results. These inconclusive results are thought to be attributed to the fact that only select parts of a whole entity are studied; how can we possibly understand a whole system if we do not account for *all* of its parts? In

contrast, complex systems analysis would suggest that multiple genes are likely to play an important role in complex chronic diseases and that many "weaker" genes (i.e., those that may be insignificant when studied in isolation with respect to the disease) when combined, may actually have a contributing effect on disease risk or state (95).

Research Significance and Objectives

The overall objective of this study is to examine the joint effect of multiple variants in folate metabolism on CVD outcome. The intermediary outcome, homocysteine, will be investigated as the primary endpoint because the metabolic disruption characterized by elevated homocysteine levels is proposed to mediate the risk of CVD. Because epidemiologic studies are limited by small sample size, and thus reduced statistical power to examine genetic interactions and their combined effects on disease outcome, we use computer simulations to study five SNPs in four candidate genes that code for enzymes that are all linked through sequential metabolic steps in homocysteine remethylation. These enzymes are either directly involved in homocysteine remethylation or indirectly linked because they provide essential substrates required for the conversion of homocysteine to methionine by MTR. Using MTR as our focal point, we also considered gene-nutrient interactions among the five variants and varying levels of folate and vitamin B12 to account for the possible effects of nutritional status on disease risk. Such an approach allows for the consideration of multiple genotype-genotype interactions and multiple genotype-nutrient interactions, capturing the complexity underlying the development of CVD. The specific research aims are as follows:

1.) To examine the interactive effects of 5 SNPs on homocysteine levels and identify genetic profiles most susceptible to elevated homocysteine levels (and by extension, elevated risk for CVD);

2.) To determine how varying levels of 5-methyl-THF (folate) and vitamin B12, both required for MTR activity, affect homocysteine levels among the different genetic polymorphism combinations.

CHAPTER 2

MODELING ALGORITHM AND PROGRAM

This study utilizes mathematical modeling to understand the overall function of the folate metabolic network as it relates to homocysteine regulation through MTR. Vast amounts of scientific literature have provided extensive detail about single reactions and single pathways that comprise folate metabolism, but as previously discussed, no study has examined the overall function of homocysteine remethylation under the influence of multiple genetic variation and the possibility of further effects created by nutritional supplementation and variation. Due to limitations in statistical power (epidemiological studies) and levels of complexity that cannot be fully captured in purely experimental studies, we utilize mathematical models to study folate metabolism as a dynamic and complex biological system.

Basic Mathematical Model for Homocysteine Remethylation

To develop models of integrated biochemical processes, it is necessary to consider the mechanisms by which biochemical information is transferred in a network. In a mathematical model of folate metabolism created by Reed et al. (96), a model is derived using standard biochemical kinetics. It has been shown to reproduce both the many known properties of folate metabolism as well as the qualitative behaviors of the folate cycle reported in experimental studies (96-97).

In our study, the effect of multiple SNPs on homocysteine regulation via MTR (highlighted subsystem in Figure 2.1) was simulated by building upon the model of folate metabolism originally published by Reed et al. and Nijhout et al. (96-97). While our research focuses primarily on the highlighted subsystem within the larger folate metabolic system depicted in Figure 2.1, our mathematical model incorporates both the folate cycle and the methionine cycle in order to account for the presence of all folate-derived, intracellular substrates as well as folate regulating enzymes. Due to

limited information on all possible SNPs in genes coding for enzymes in the folate and methionine cycles, we have made some parameter and substrate values constant in our study and have focused primarily on genetic and nutrient outcomes related to genes and enzymes in our highlighted subsystem of interest. The values, expressed in micro Molar, µM, that we have kept constant are given in Table 2.1 and are based on information reported in the literature.

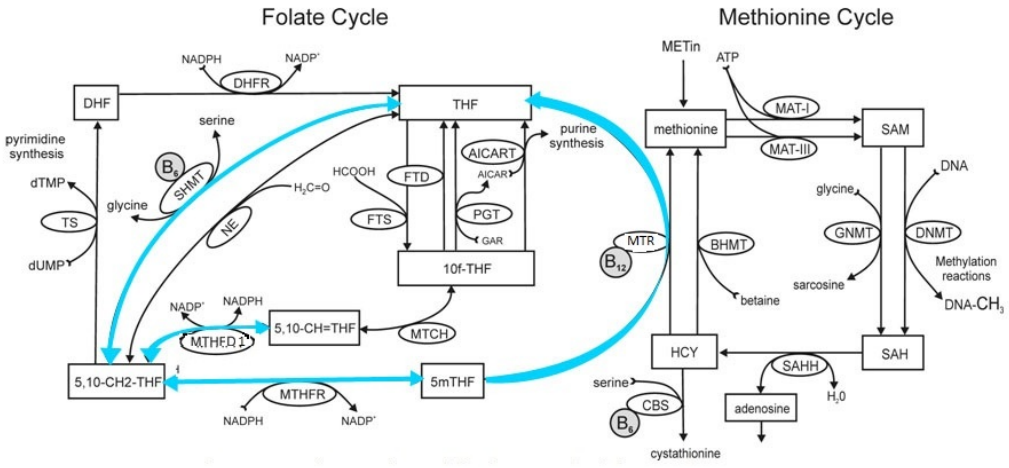


Figure 2.1: Folate and Methionine Cycle, adapted from Reed et al. (96). Substrates are represented by rectangular boxes, enzymes in ovals and vitamin cofactors in circles

Table 2.1: Concentrations of Substrates Used in Model that are held constant (units: μM)

Substrate	Concentration	Reference
glycinamide ribonucleotide [GAR]	10	98
aminoimidazolecarboxamide ribonucleotide [AICAR]	2.1	98-100
nicotinamide adenine dinucleotide phosphate [NADPH]	50	98,101
Glycine [GLY]	1850	98,99,102
Serine [SER]	468	98,99,102
Betaine [BET]	50	96
Formate [HCOOH]	500	100,102
Formaldehyde [$H_2C = O$]	500	96
deoxyuridine monophosphate [dUMP]	20	98-100

Our current model of the folate metabolic cycle is represented by ten differential equations and various enzyme velocity equations that describe folate substrate concentration over time. For each enzyme, the K_m is given in μM and the V_{max} and the enzyme velocities of the reactions, V, are expressed in μM/hr. The abbreviated notation used to describe our metabolic system is as follows:

5mTHF = 5-methyltetrahydrofolate
THF = tetrahydrofolate
DHF = dihydrofolate
CH_2F = 5-10-methylenetetrahydrofolate
CHF = 5-10-methenyltetrahydrofolate
10fTHF = 10-formyltetetrahydrofolate
MET = methionine
SAM = S-adenosylmethionine
SAH = S-adensylhomocysteine
HCY = homocysteine
Met_{in} = influx of methionine into the system (μM/hr)
$F_{in/out}$ = influx and outflux of folate

Initial concentrations for all variables in our model are given in Table 2 and are based on steady-state values found in the literature.

Table 2.2: Initial Substrate Concentrations Used in Models (units: µM)

Substrate	Concentration	Reference
MET	48	96
SAM	64.42	96
SAH	13.04	96
HCY	1.11	96
CH_2F	0.90	96
5mTHF	5.16	8,9
THF	8.01	96
DHF	0.03	97, 98
CHF	1.12	96, 97
10fTHF	5.93	98, 100, 103,104

Since substrate concentration dictates the velocity of reactions catalyzed by enzymes, the following differential equations were used to describe the change in substrate concentration over time:

$$\frac{d}{dt}[5mTHF] = V[MTHFR] - V[MS] + Ftn - Fout$$

$$\frac{d}{dt}[THF] = V[MS] - V[FTS] + V[PGT] + V[AICART] + V[DHFR] - V[SCHMT] - V[NE] + V[FTD]$$

$$\frac{d}{dt}[DHF] = V[TS] - V[DHFR]$$

$$\frac{d}{dt}[CH2f] = V[SHMT] + V[NE] - V[TS] - V[MTD] - V[MTHFR]$$

$$\frac{d}{dt}[CHF] = V[MTD] - V[MTCH]$$

$$\frac{d}{dt}[10fTHF] = V[MTCH] + V[FTS] - V[FGT] - V[AICART] - V[FTD]$$

$$\frac{d}{dt}[MET] = V[BHMT] + V[MS] - V[MATI] - V[MATIII] + Metin$$

$$\frac{d}{dt}[SAM] = V[MATI] + V[MATIII] - V[GNMT] - V[DNMT]$$

$$\frac{d}{dt}[SAH] = V[GNMT] + V[DNMT] - V[SAAH]$$

$$\frac{d}{dt}[HCY] = V[SAAH] - V[CBS] - V[BHMT] - V[MS]$$

The velocities of the reactions for each enzyme in our model are given in Table 2.3.

Table 2.3: Kinetic Parameter Values Used in Model

Parameter	Model	Reference
AICART		106-109
$K_{m,s\ (AIRCARP)}$	100	
$K_{m,F\ (10f\text{-}THF)}$	5.9	
V_{max}	45,000	
BHMT		110-112
$K_{m,F(HCY)}$	12	
$K_{m,S(BET)}$	100	
V_{max}	1125	
CBS		113-115
K_m (HCY)	1000	
V_{max}	90,000	
DHFR		106,107,97,116
$K_{m,s\ (NADPH)}$	4	
$K_{m,F\ (DHF)}$	0.5	
V_{max}	50	
DNMT		117
K_m	1.4	
Ki	0.84	
V_{max}	180	
FTD		118
$K_{m,F\ (10f\text{-}THF)}$	20	
V_{max}	14,000	
FTS		106,108
$K_{m,s\ (HCOOH)}$	43	
$K_{m,F\ (THF)}$	3	
V_{max}	2000	
GNMT		119,105, 120
K_m	63	
Ki	10.8	
V_{max}	288	
MAT-I		121
K_m (MET)	41	
V_{max}	260	
MAT-III		121,122
K_m (MET)	300	
V_{max}	220	
MTCH (reversible)		106-109
$K_{m,F1\ (CHF)}$	250	
$K_{m,F2\ (10f\text{-}THF)}$	100	
V_{max1}	800,000	
V_{max2}	20,000	
MTHFD1 (reversible)		106,109,97,123
$K_{m,F1\ (CH2F)}$	2	

Table 2.3 (Continued)

$K_{m,F2\ (CHF)}$	10	
V_{max1}	200,000	
V_{max2}	594,000	
MTHFR		124-128
$K_{m,\ s(NADPH)}$	16	
$K_{m,F\ (5,10\text{-}CH2\text{-}THF)}$	50	
V_{max}	5000	
MTR		129-131
$K_{m,F\ (5mTHF)}$	25	
$K_{m,F\ (HCY)}$	0.1	
V_{max}	500	
NE of THF by 1st order mass action		132-133
k_1	0.15	
k_2	12	
PGT		106,107,134,135
$K_{m,s\ (GAR)}$	520	
$K_{m,F\ (10f\text{-}THF)}$	4.9	
V_{max}	16,200	
SAHH		136
K_m (SAH)	10	
K_m (HCY)	1	
V_{max1}	5000	
V_{max2}	5000	
SHMT (reversible)		106-109,97,127,137-139
$K_{m,s1\ (Serine)}$	600	
$K_{m,F1\ (THF)}$	50	
$K_{m,s2(Glycine)}$	10,000	
$K_{m,F2(CH2\text{-}F)}$	3,200	
V_{max1}	40,000	
V_{max2}	25,000	
TS		106,107,97,140, 141
$K_{m,dUMP}$	6.3	
$K_{m,CH2F}$	14	
V_{max}	5000	

Time is given in hours, h, and concentrations are expressed in μM

Many of the velocity equations are assumed to behave dependently on their substrates as dictated by Michaelis-Meten kinetics (96). Thus, the rate of enzymatic activity for irreversible reactions (i.e., reactions catalyzed by MTR) is defined as

$$V[Enzyme] = Vmax\left[\left(\frac{[S]}{Km,s + [S]}\right)\left(\frac{[F]}{Km,F + [F]}\right)\right]$$

and for reversible reactions (i.e., reactions catalyzed by MTHFD1 and cSHMT),

$$V[Enzyme] = Vmax1\left[\left(\frac{[S1]}{Km,s1 + [S1]}\right)\left(\frac{[F1]}{Km,F1 + [F1]}\right)\right] Vmax2\left[\left(\frac{[S2]}{Km,s2 + [S2]}\right)\left(\frac{[F2]}{Km,F\ 2 + [F2]}\right)\right]$$

where S = nonfolate substrate concentration

F = folate substrate concentration

In the global folate metabolic network-- and not in our highlighted subsystem of interest- the reaction between CH_2F and THF that is accounted for in our model is non-enzymatic. Because of this we expect the reaction to follow a mass action rate law of pseudo first-order where k_1 and k_2 are rate constants:

$V[NE] = k_1\,[THF][H_2C{=}0] - k_2\,[CH_2F]$

The remaining velocity equations are presented individually, as they do not strictly adhere to general Michaelis-Menten kinetics (96); we illustrate the kinetics of each [enzyme] below as originally published by Reed et al. (96) such that when parameter values (i.e., K_m and V_{max}) given in Table 2.3 for respective enzymes are used in the equations, the kinetics are Michaelis-Menten. Concentrations of SAM and

SAH are included as regulatory methods (either activating or inhibiting an enzyme) and scaling factors are used so that the value of regulation equals 1 for our models since our initial methionine input rate (met_{in}) is set at 100µmol/(L hr). The input rate for the starting methionine level is chosen based on the work of Storch et al. (105) who found that over a 24 hour period, mean methionine levels in the human liver is approximately 100 µmol/(L h) after accounting for fasting and feeding states during a typical 24 hour period.

$$V[BHMT] = e^{-.0021([SAM]+[SAH])} e^{.0021(77.2)} \left(\frac{Vmax[HCY][BET]}{(Km1 + HCY)(Km2 + [BET])}\right)$$

$$V[CBS] = \left(\frac{Vmax[HCY]}{Km + [HCY]}\right)\left(\frac{1.2([SAM] + [SAH])^2}{30^2 + ([SAM] + [SAH])^2}\right)$$

$$V[DNMT] = \frac{Vmax[SAM]}{Km\left(1 + \frac{SAH}{Ki}\right) + [SAM]}$$

$$V[GNMT] = \left(\frac{Vmax[SAM]}{Km + [SAM]}\right)\left(\frac{1}{1 + \frac{[SAH]}{Ki}}\right)\left(\frac{4.38}{0.35 + [5mTHF]}\right)$$

$$V[MAT\text{-}I] = \left(\frac{Vmax[MET]}{Km + [MET]}\right)(0.23 + (0.8)e^{-(.0026)[SAM]})$$

$$V[MAT\text{-}III] = \left(\frac{Vmax[MET]^{0.86}}{Km + [MET]^{0.86}}\right)\left(1 + \frac{(7.2)[SAM]^2}{Ka^2 + [SAM]^2}\right)$$

$$V[MTHFR] = \left(\frac{Vmax[CH2F][NADPH]}{(Km1 + [CH2F])(Km2 + [NADPH])}\right)\left(\frac{(6.1)(10)}{10 + [SAM] - [SAH]}\right)$$

Interactive Effects of Multiple Genetic Variants on Homocysteine Remethylation

Building upon the basic folate metabolic model, we developed a mathematical model that accounts for the interactive effect of five unique SNPs in genes coding for enzymes involved in the methylation of homocysteine by MTR. The genetic effects resulting from each of the five SNPs are expressed with a scalar value that serves as a multiplier to the SNP's corresponding enzyme velocity equation. Since a person can have one of any three possible genotypes for each of the five genetic polymorphisms (i.e., homozygous dominant, heterozygous or homozygous recessive), a total of 243 possible genetic combinations, and subsequent graphs, are created. Based on findings in the literature, the homozygous dominant genotype for each of the five SNPs is classified as the "wild type" genotype (142-147). Enzyme rate for individuals with the "wild type" genotype for a given SNP is assumed to be fully functional at 100% activity. To date, the exact rate of activity for each enzyme for a particular genotype, with the exception of MTHFR, has not been determined experimentally or published in the literature. Without such information, we approximate that the heterozygous and homozygous recessive genotypes reflect a 60% and 30% enzyme activity rate, respectively. These hypothesized reductions in enzyme activity rate for MTR, MTHFD1 and cSHMT are motivated by biological hypotheses that assume enzyme kinetics and the number of variant allele copies have a dose-response relationship: having a single variant allele corresponds to a slightly reduced enzyme activity level and having double variant allele copies corresponds to an even greater reduction in enzyme activity level. In MTHFR, the observed functional effect resulting from the *MTHFR* 677 C→T polymorphism is 60% enzyme activity for heterozygotes (CT) and 30% in homozygote variants and 90% (AC) and 68% (CC) enzyme activity for *MTHFR* 1298 A→C heterozygote and homozygous variant alleles, respectively (106-

108, 121, 123, 128, 137, 139-141, 148-153). Table 2.4 shows the full assignment of percentages for the SNPs.

Table 2.4: List of Functional Effects on Enzyme Activity by Polymorphism

SNP	% of wild-type activity
MTHFR 677 C→T	
CC	100
CT	60
TT	30
MTHFR 1298 A→C	
AA	100
AC	90
CC	68
MTHFD1 1958 G→A	
GG	100
GA	60
AA	30
MTR 2756 A→G	
AA	100
AG	60
GG	30
cSHMT 1420 C→T	
CC	100
CT	60
TT	30

The Role of Gene-Nutrient Supplementation in Homocysteine Remethylation

The remethylation of homocysteine through MTR involves several nutrient cofactors, particularly vitamin B12 and folate. To determine if nutrient levels influenced the relationship between genetic variation and homocysteine levels by effect modification, we varied the concentration of folate and vitamin B12 for each of the 243 genetic profiles. In our model, folate enters and leaves the cell as 5mTHF. The equations for F_{in} and F_{out}, both expressed in μM/hr, is:

$$F_{in} = (\text{Steady state folate concentration})(0.0008)/24$$

$$F_{out} = \alpha([5mTHF]) \quad , \alpha = 0.0013/h$$

According to Nijhout et al.(97), these expressions assume that the various folate pools in the body are in equilibrium and that the intracellular folate pool is depleted at the same rate as the total body folate pool which was approximated at 0.8% per day. By choosing 20 μM for steady-state folate concentration, the concentrations and values of other folate metabolites as listed in Table 2.2 stay within ranges that have been observed in studies reported in the literature. Thus, when steady state folate concentration is set at 20 μM, $F_{in} = 0.0067$. Assuming that the output from the cytosol is a first order rate process at steady state, F_{out} must also equal to 0.0067. The rate constant α is determined to be 0.0013 since the steady state concentration of 5mTHF is 5.16 μM (Table 2.2).

The effects of a vitamin B12 and folate dose were achieved by applying a scalar multiplier of 14 to *V[MTR]* and 100 to *V[MTHFR]*. The scalar multiplier for vitamin B12 corresponds to the findings reported in the literature of a 14-fold increase in MTR activity in liver cells supplemented with vitamin B12 (97). Epidemiological studies have shown that high dietary folate supplementation attenuates elevated homocysteine levels observed among individuals with *MTHFR* genetic variants (154-157). In our model, we assumed that there was a 100 fold increase in MTHFR activity

rate as a result of folate supplementation on a cellular level. Vitamin B12 and folate degradation in the body was considered to be negligible in our models because of the large half-life of vitamin B12 (400 days) and folate (100 days) (158,159). Since experimental studies have also shown that changes in MTR activity can be observed as early as two hours following vitamin B12 addition and that activity plateaus within ~24-48 hours after nutritional dosing (158), our models simulate nutrient supplementation and its effects on homocysteine concentration and steady state kinetics over a 48 hour period.

Program structure

Our model is written in Matlab and consists of four functions: *ODE*, *RHS*, *V_ENZYME* and *COMBO_EFFECT*. The *ODE* function is responsible for solving the differential equations and displaying the results for homocysteine and calls information from the *RHS* function. The *RHS* function defines the differential equations in terms of enzyme velocities which are subsequently defined in the *V_ENZYME* function. In addition to the velocity equations, the *V_ENZYME* function also includes associated modifiers for the effects of the SNP combinations and vitamin B12. As illustrated by Figure 2.2, the first step is where the user assigns the model parameters. The model parameters include the initial folate and non-folate substrate concentrations, folate steady state influx, vitamin B12 dosage, the profile of SNP combinations to examine, simulated time and time step. After values for all input variables have been assigned and the program is initiated, the differential equations in *RHS* are initially solved from t = 0 to the first time step assigned. The resulting folate substrate concentrations then replace the previously assigned initial folate substrate concentrations and the program then undergoes another iteration, using the new folate concentration. This loop continues until the assigned simulated time has been reached.

The annotated Matlab code outlining each step needed to generate our models is found in Appendix A1-A4.

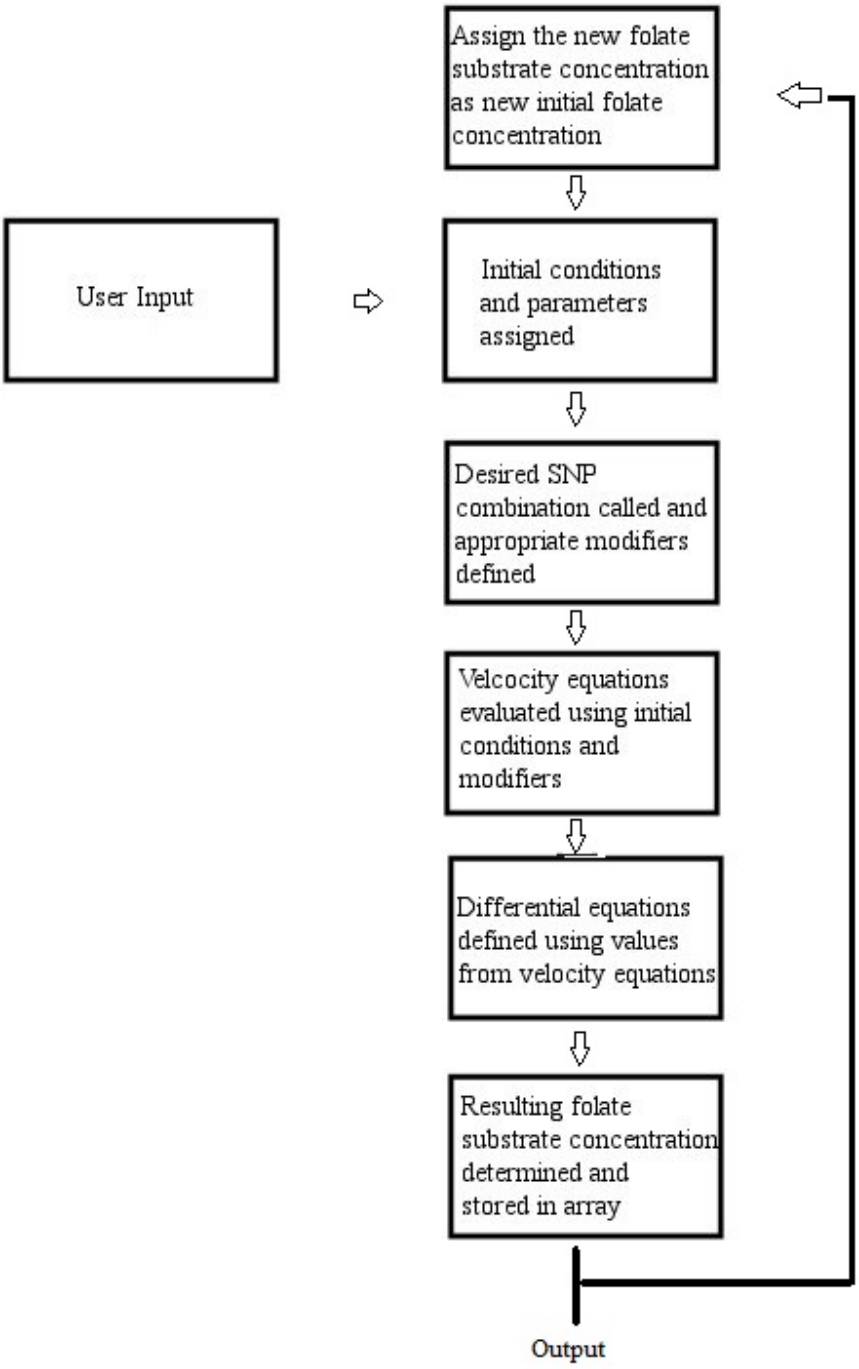


Figure 2.2: Flow chart of order of program processes

CHAPTER 3

FINDINGS AND CONCLUSIONS

No Supplementation

We modeled 150 scenarios for each of the 243 genotype combinations possible among our five single nucleotide polymorphisms of interest. For each of the 243 genotype profiles, we computed mean homocysteine levels (μM).

Lowest homocysteine concentration

Without any nutrient supplementation, our simulations suggest that individuals with the *MTHFR* 677 *TT*, *MTHFR* 1298 *CC*, *MTHFD1* 1958 *GG*, *MTR* 2756 *AA* and *cSHMT* 1420 *TT* genotypes have the lowest concentration of homocysteine (4.5μM). In this genotype, only the *MTHFR* gene carries the mutated genotypes, *MTHFR* 677 *TT* and *MTHFR* 1298 *CC*, resulting in decreased function. The remaining enzymes are functioning at full capacity in this model, with the exception of cSHMT.

The consequence of this genotype appears to be that:

1. Adequate substrate is delivered to MTHFR through the optimal action of MTHFD1;
2. With no mutation observed in the *MTR* 2756 A→G gene, MTR is able to remove all 5-methylTHF substrate, driving the reactions of the homocysteine remethylation cycle in the forward direction.
3. Because the products of MTHFR activity are quickly removed from the system, while at the same time there is a relative glut of products from MTHFD1 action, the reaction pathway for MTHFR → MTR is not reversed to favor the buildup of 5,10-methyleneTHF.

As a result, homocysteine is methylated efficiently in this scenario.

Previous single gene studies of the *MTHFR* 677 C→T mutation have yielded equivocal results with respect to homocysteine status. The mechanism presented here may explain the lack of positive findings (i.e., elevated homocysteine levels) in the presence of the MTHFR polymorphism. It should be noted that there may be a publication bias in that negative results with this polymorphism may be discounted, as a simplistic consideration of this pathway would suggest that a polymorphism in the MTHFR gene would yield a positive result.

Highest homocysteine concentration

Simulation results show that among all possible 243 genotype profiles, the genotype group with the highest homocysteine concentration (11.9 μM) has the following profile: *MTHFR* 677 *TT*, *MTHFR* 1298 *AA*, *MTHFD1* 1958 *AA*, *MTR* 2756 *GG* and *cSHMT* 1420 *CC*.

With reduced function in MTHFD1 due to polymorphisms in *MTHFD1* 1958 G→A, the generation of 5,10-methyleneTHF is decreased, leaving only a small pool of substrate available for MTHFR to catalyze the reaction to make 5-methylTHF for MTR use. With diminished substrate levels for MTR due to polymorphisms in the *MTHFD1* 1958 G→A and *MTHFR* 677 C→T genes, normal MTR function is reduced, leading a build-up of homocysteine levels. The addition of a polymorphism in *MTR* 2756 A→G, causing reduced MTR activity would therefore lead to an even greater increase in homocysteine concentration, which is what we observed in our highest homocysteine group.

This result is not surprising, given that the series of mutations in the first 4 enzymes would be expected to decrease the delivery of single carbon moieties to homocysteine. This is an example of where the mutation of the MTHFR enzyme was associated with elevated homocysteine levels, in contrast with the previous model, and in synch with expectations. Taken together these examples illustrate the importance of

modeling a complete set of gene-gene interaction rather than relying on single gene studies.

The model with all the mutated polymorphisms, including *MTHFR* 1298 CC yields a low homocysteine level (5.8μM). It seems at first surprising that the genotype with every possible mutation in every enzyme would result in low rather than high levels of homocysteine. However it is clear from the examples given above that it is not the presence of mutations per se that influence homocysteine levels, but rather an imbalance in the activities of MTHFR vis-à-vis the remaining enzymes. The *MTHFR* 1298 *CC* mutation debilitates this enzyme even further, with the consequence that, relatively speaking it is more inefficient than the other enzymes. This increased inefficiency results in a pattern of relative function that is virtually the same as the pattern where only the *MTHFR gene* carries the mutation. As we saw earlier, this pattern yields a low level of homocysteine.

It is clear then, that MTHFR serves as a regulator for determining whether the single-carbon units are passed either to the methionine regeneration pathway or to the thymidylate pathway. Contrary to what may be expected a priori, in this model a polymorphism in the *MTHFR* gene without polymorphisms in the other enzymes, or a double polymorphism in *MTHFR* coupled with polymorphisms in the other enzymes, actually leads to a better balance between substrate and product than does a lack of *MTHFR* polymorphism. The explanation for this finding lies in the fact that, as the model was constructed, the MTHFR enzyme can catalyze the reaction in both directions, towards MTR and towards the thymidylate pathway. By slowing down throughput towards the MTR pathway with a MTR function high relative to MTHFR, MTHFR has to function unidirectionally, that is, towards the production of methionine.

Finally, an in vitro study directly assessing the effect of *MTHFR* 1298 A→C on enzyme activity and thermolability concluded that the *MTHFR* 677 C→T variant was more deleterious than the alanine variant of *MTHFR* 1298 A→C (54), suggesting that the role of *MTHFR* 1298 A→C on MTHFR enzyme activity is minor compared to that of *MTHFR* 677 C→T. Overall, the evidence to date is suggests that the effect of *MTHFR* 1298 A→C is not observed to have an effect on clinical outcomes unless there is a reduced folate status or there is also a variant allele at the 677 nucleotide (*MTHFR* 677 C→T).

Effect of folate supplementation

The addition of folate into the system has no effect on homocysteine levels. Because folate acts at each point in these pathways, its levels cannot alter the balance in the pathways caused by the presence or absence of polymorphisms. The model as constructed assumes normal levels of folate in the unsupplemented condition. To see any effect of additional folate on these pathways, it would be important to run the model under folate-deficient conditions.

Effect of vitamin B12 supplementation

By contrast with folate, vitamin B12 occurs at only one point in the homocysteine remethylation pathway, namely at the level of MTR. In this model, vitamin B12 was designed to increase the activity of MTR. Any increase in MTR activity would be expected to accelerate the transfer of single carbon moieties to homocysteine, thus decreasing homocysteine levels. This is indeed what was found for each of the genotypes (Appendix A5). However, vitamin B12 cannot, at least in this model system, bring homocysteine levels down to normal.

Limitations of the study

The models in this study are constructed to simulate the effects of mutations on enzyme activity by changing enzyme kinetic rates. Except for *MTFHR* 677 C→T, we

have assumed that mutations have a dose-response effect on enzyme availability and overall activity levels. The true effects of the *MTR*, *cSHMT* and *MTHFD1* polymorphisms could be better represented in our models if such specific information on their effects were known. However, our models are created in such a way that they can be updated when new information on mutational effects become available.

Our models do not account for other linked cycles in the folate metabolic network. We have focused solely on the homocysteine remethylation pathway. The overall cellular folate concentrations of our current model may not fully predict the function of a given pathway since folate metabolism can be compartmentalized in the cell by substrate channeling through linked cycles such as those for purine or thymidylate syntheses.

We focused only on folate and vitamin B12 supplementation in our models. Our simulations assumed that no nutritional deficiency existed before supplementation. Differences in homocysteine levels among those with low nutrient status were not explored and could possibly be useful for better understanding the interaction of vitamin cofactors with their respective enzymes.

Conclusions and suggestions for further research

By examining homocysteine remethylation as a complete pathway, our mathematical models were able to capture the combined effects of linked metabolic steps on homocysteine concentration. This approach led to the key finding that having double variants for all possible polymorphisms in a pathway does not necessarily equate to the most deleterious effects. It also illustrates how pathways have built-in regulatory mechanisms that researchers might not be able to account for when taking a single candidate gene approach to studying disease outcome.

A key advantage of our simulations is that they provide for quick and easy investigation of variation in multiple inputs (i.e., gene-gene and gene-nutrient

interactions) that cannot be explored in traditional epidemiology studies. Our models can account for an unlimited number of variables and can be expanded to represent larger metabolic networks. Future research can expand upon the work presented by linking other metabolic cycles that are associated with substrates or enzymes already found in homocysteine remethylation (i.e., purine or thymidylate syntheses). Additionally, research can be done to understand how equilibrium is attained by certain genotype combinations. This could also be expanded by seeing how nutrient supplementation further modifies how steady state is achieved.

In conclusion, our mathematical modeling of homocysteine remethylation provides a new tool for investigating the effects of genetic variation and nutrient effects in one carbon metabolism. We anticipate that our model will serve as an example of how simulations can help advance the growing idea that disease treatment can be personalized by examining an individual's unique genetic and nutritional profile.

APPENDIX

A1: ODE Annotated Matlab Program

```
%This program models the folate and methionine cycle.

%Modifiable settings are the following:
%(1) The set of SNP combos one wishes to model.
%This is under the ODE function under the variable
%s at line 42.

%(2) Length of time to simulate. This is under the
%"tend" variable under the ODE Function at line 57.
%The units are in hours. In addition, one can also
%change how many steps the program takes to get from
%t=0 to t=tend.

%(3) Initial folate concentration. It is under the
%RHS function at line 34.  For our model, the
%default is 100uM.

%(4) The rate of methionine into the system
%(to simulate met loading). This is found in
%the RHS function at line 33. Units are uM/hr.

%(5) Vitamin B12 supplementation. When there is
%supplementation, 3689uM of  B12 is added into the
%system. This is done by manipulating the variable
%"Vitamin_Dose" in the Enzyme function at line 21.
%Just change the value to equal 1 for B12 loading
%or 0 for no loading.

clear global s;
clf('reset');
tic
    global s;
    %sets s as a global variable. This variable is
    %used in the “Combo_Effect” function
    HCY=zeros(121,6);
    THF=zeros(121,6);
    %Line 35 and 36 declares two empty 101x6
    %matrices named HCY and THF that the following
```

```
%code will fill with data points
HCY_col=1;
THF_col=1;
    for s = (1:6);
     %This here starts the for loop that causes
     %the program to go through SNP combos 1-6.
     %“for s= (1:6) means “for s (the row of
     % vectors of the combination matrix)=1
     %through 6. To change the %set to 7-12,just
     %set s equal to "7:12" instead of "1:6".

        values0= [5.16, 8.01, 0.03, 0.90, 1.12,
                  5.93, 48, 64.42, 13.04, 1.11];
            %These are the initial folate substrate
            %concentrations in uM in the order:
       %[5mTHF, THF, DHF, CH2F, CHF, 10fTHF, MET,
        SAM, SAH, HCY, HCY]

        tend= 0.5;
        %tend is the total time in hours that we
         are modeling over

        tspan=[0:tend/120:tend];
        %tspan defines the time interval that
        %will be modeled, as well as how many
        %time steps will be taken. It is
         currently set to start at O hour and go
         to tend hours at tend/120 time steps

        options = odeset('NonNegative', [1:6]);
        %Here we're setting some parameters for
        %our ode solver. Note -- NonNegative
        %means that whenever the solution
        %approaches zero, MatLab will be extra
        careful in choosing the next time step
        for solving the ODE

        [T,X] = ode45(@RHS,tspan,values0,options);
        %This is where we call the ordinary
        %differential equation (ODE) solver. The
        %differential equations are called from
        %the RHS function and are solved with the
```

```
81              %initial values, values0, as well as,
82              %with respect (wrt) to the options
83              %defined in line 51 The solutions are
84              %then stored in the matrix X
85
86              HCY(:,HCY_col)=X(:,end);
87              THF(:,THF_col)=X(:,2);
88              %Line 86 assigns the s column vector of
89              %HCY to equal the end column vector
90              %(the tenth one in this case) of matrix X
91              HCY_col=HCY_col+1;
92              THF_col=HCY_col+1;
93              %Line 91 and 92 tell the code to record
94              %the values in the next column to the
95              %right
96          end
97
98  %In Summary, lines 42-92 define what the ODE function
99  %does for each SNP %combination that is defined in the
100 %“for s=” statement of line 42. In this annotated code,
101 %the ODE function would run 6 times because there
102 %are 1:6 combination codes that have been defined
103 %by the user.
104
105             plot(T,HCY(:,1),'-or')
106             %Line 105 plots the 1st column vector of
107             %matrix HCY.
108             hold all
109             plot(T,HCY(:,2),'-+g')
110             plot(T,HCY(:,3),'-*b')
111             plot(T,HCY(:,4),'-vc')
112             plot(T,HCY(:,5),'-sk')
113             plot(T,HCY(:,6),'-om')
114             hleg = legend('1','2','3','4','5','6',
115                    'Location','NorthEastOutside');
116             %Line 114 fills out the legend of the plot.
117             % The key for whatthese numbers mean are
118             %in the “Combo_Effect” function. The
119             %legend has to be changed manually every
120             %time to move from one set of 5 SNP
121             %combinations to another.
122             xlabel('hours');
```

```
123             ylabel('[HCY]uM');
124             HCY
125             THF
126             %Lines 124-125 print out the values for
127             %each data point. Each  column maps to a
128              %graph. Thus, the 1st column is the top
129             %graph on the legend.
130
131             str = sprintf('SetX_noB12_folate 20')
132         %Line 131 prints out which set is currently
133         %being modeled. It serves as a manual and
134         %internal check; each time the user runs
135         % the simulation, the value within sprintf()
136         %should be manually changed so when the output
137         %is displayed in Matlab, the combination and
138         %modifiers used are also displayed and
139         %cross-referenced.
140         toc
141         %the tic toc command in this function directs
142         %the program to report the total time it
```

A2: RHS Annotated Matlab Program

```
function xdot = RHS(t,x)

%The RHS function is where all of the ODEs that are
%solved in the “ODE function” are defined.

Polymorphism_effect= Combo(1);
%Line 6 creates the variable “Polymorphism_effect”
%which stores the data called from combo(1).
%Combo(1) is an address that is specific to a SNP
%combination found in the “Combo_Effect” function.
%The address function is necessary when calling
%numbers from a separate function. The 1 in the
%parenthesis is just a placeholder for calling the
%data from the “Combo_Effect” function, so it can
%take on any value defined in “Combo_effect”.

Velocity = Enzyme(x,Polymorphism_effect);
%To solve the differential equations, the velocity
%values for each enzyme in %the system must be called
%in. Line 17 calls in the “V_Enzyme” function with
%the parameters x and Polymorphism_effect, where x
%is our initial folate and methionine substrate
%concentrations for the first run through of the
%folate %and methionine cycle. With each subsequent
%iteration, x changes its value to the new resulting
%concentration of folate and methionine substrates.

%SETTINGS ARE HERE

%The influx of methionine (uM/hr) and folate (uM/hr)
%into the cycle can be modified in lines 27 and 28,
%respectively.
Metin = 0;
Folate_in = 100;

%In the following code, xdot refers to
%d[substrate concentration]/dt

xdot=zeros(10,1);
```

```
40
41  % 5mTHF
42  xdot(1)= Velocity(1)-Velocity(7) +
43           ((Folate_in*0.008)/24) - (0.0013*x(1));
44
45  % THF
46  xdot(2)= Velocity(7)-Velocity(5)+Velocity(3)+
47           Velocity(4)+Velocity(2)-Velocity(8)-
48           Velocity(11)+Velocity(6);
49
50  % DHF
51  xdot(3)= Velocity(12)-Velocity(2);
52
53  % CH2F
54  xdot(4)= Velocity(8)+Velocity(11)-Velocity(12)-
55           Velocity(9)-Velocity(1);
56
57  % CHF
58  xdot(5)= Velocity(9)-Velocity(10);
59
60  % 10fTHF
61  xdot(6)= Velocity(10)+Velocity(5)-Velocity(3)-
62           Velocity(4)-Velocity(6);
63
64  % MET
65  xdot(7)= Velocity(18)+Velocity(7)+Metin -
66           Velocity(13)-Velocity(14);
67
68  % SAM
69  xdot(8)= Velocity(13)+Velocity(14)-Velocity(15)-
70           Velocity(17);
71
72  % SAH
73  xdot(9)= Velocity(15)+Velocity(17)-Velocity(19);
74
75  % HCY
76  xdot(10)= Velocity(19)-Velocity(16)-Velocity(18)-
77            Velocity(7);
78  end
```

A3: V_Enzyme Annotated Matlab Program

```
function V_Enzyme = Enzyme(x,y)
     %This function defines all of the values used in
     %the RHS function where the ODEs are stored. It
     %also takes into account the modifying elements
     %for the different SNP combinations from the
     %"Combo_Effect" function. The %parameters in the
     %parentheses, x and y, represent the folate
     %substrate concentration and the SNP modifiers,
     %respectively.

    Vmax = [5000, 5000, 16200, 45000, 3000, 3300,
            500, 40000, 25000, 200000, 594000,
            800000, 20000,5000, 260, 220, 288,
            90000, 180, 1125, 5000, 5000];

    %Vmax is the Michaelis-Menten enzyme kinetic
    %parameter. Time is in hours, concentrations are
    %in uM and the numbers are in the order used for
    %the respective enzymes in the equations below:

    Vitamin_Dose = 0;
    %The user can choose to modify whether or not
    %supplementation by changing the value following
    %"vitamin_dose=". Vitamin_Dose can take a binary
    %value of either 0 or 1 where 0 means there is
    %no vitamin B12 supplementation and 1 means there
    % is vitamin B12 supplementation. When there is
    %vitamin B12 supplementation, 3689uM of vitamin
    %B12 is added to the system. This value is
    %selected for the concentration of vitamin B12
    %supplementation because the DRI  states that
    %normal B12 absorption rate and reabsorption
    %rate is   0.1% per day and 3689 uM is the
    %highest amount of vitamin B12 that can be added
    %to a system depleted of vitamin B12 before
    %vitamin B12 retention occurs (i.e. flux in does
    %not equal flux out when B12 concentration is
    %greater than 3689uM).

```

```
40      Vitamin_Effect = 0;
41      %The value for Vitamin_Effect is dependent on
42      %the value assigned for Vitamin_Dose. If there
43      %is vitamin B12 supplementation (Vitamin_Dose=1)
44      %, the literature states that the effect of the
45      %supplementation on MTR activity increases by a
46      %factor of 14 (lines 52-53). If there is no
47      %supplementation then Vitamin_Effect=1 because
48      %MTR activity stays the same as if a person has
49      %their steady state concentration of
50      %vitamin B12 (line 55).
51
52      if Vitamin_Dose == 1
53          Vitamin_Effect = 14;
54      else
55          Vitamin_Effect = 1;
56      End
57
58
59      nf_pool = [10, 2.1, 50, 1850, 468, 50, 500,
60                 500, 20, Vitamin_Effect];
61
62      %nf_pool is a list of non-folate derived
63      %substrates that are present in our system. In
64      %our simulations, these values are kept constant
65      %and the constants are steady state
66      %concentrations in uM. Theoretically, these
67      %values can be modified, but they are not for the
68      % purposes of this model.order:GAR, AIRCAR,
69      %NADPH, GLY, SER, BET, HCOOH, H2C=O, DUMP,
70      %Vitamin B_Effect
71
72      V_Enzyme = zeros(19,1);
73      %Line 72 declares a matrix V_Enzyme with the
74      %zero matrix that has 19 rows and 1 column
75
76      %All 19 of these equations below were defined
77      %by Reed et al. The equations all follow
78      %Michaelis-Menten kinetics.
79      %Order:
80      %(1)MTHFR, (2)DHFR, (3)PGT, (4)AICART, (5)FTS,
81      %(6)FTD, (7)MS, (8)SHMT, (9)MTD, (10)MTCH,
```

```
    %(11)NE, (12)TS,(13)MAT-I, (14)MAT-II, (15)GNMT,
    %(16)CBS, (17)DNMT, (18)BHMT,(19)SAHH

%Below are the rate reactions for the enzymes listed
%in lines 80-83).
    V_Enzyme(1)= y(1)*(Vmax(1)*
    ((nf_pool(3)/(16+nf_pool(3)))*((x(4)/(50+x(4)))))
    *(6.1/(10+x(8)-x(9))));
    %Recall that "y" was our input from the SNP
    %modifier (see annotation for line 1 of V_Enzyme
    % function). Here is where "y" does the
    %modification.
    % y(1) defines the first element in the row
    %vector
    %y that is sent over to this function.

    V_Enzyme(2)= Vmax(2)*((nf_pool(3)/(4+nf_pool(3)))
                 *((x(3)/(0.5+x(3)))));
    V_Enzyme(3)= Vmax(3)*((nf_pool(1)/
                 (520+nf_pool(1)))*((x(6)/
                 (4.9+x(6)))));
    V_Enzyme(4)= Vmax(4)*((nf_pool(2)/
                 (100+nf_pool(2)))*((x(6)/
                 (5.9+x(6)))));
    V_Enzyme(5)= Vmax(5)*((nf_pool(7)/
                 (43+nf_pool(7)))*((x(2)/
                 (10+x(2)))));
    V_Enzyme(6)= Vmax(6)*((x(6)/(0.9+x(6))));
    V_Enzyme(7)= Vitamin_Effect*(y(3)*((Vmax(7)
                 *((x(10))/(0.1+x(10))))
                 *((x(1)/(25+x(1))))));
    V_Enzyme(8)= y(4)*((Vmax(8)*((nf_pool(5)/
                 ((600+nf_pool(5))))*((x(2)/
                 (50+x(2))))))-(Vmax(9)*((nf_pool(4))
                 /(10000+nf_pool(4)))*((x(4))/
                 (3200+x(4)))));
    V_Enzyme(9)= y(2)*((Vmax(10)*((x(4)/(2+x(4)))))-
                 (Vmax(11)*(x(5)/(10+x(5)))));
    V_Enzyme(10)= (Vmax(12)*((x(5)/(250+x(5)))))-
                  (Vmax(13)*(x(6)/(100+x(6))));
    V_Enzyme(11)= 0.15*x(2)*nf_pool(7)-12*x(4);
    V_Enzyme(12)= Vmax(14)*((nf_pool(9)/
```

```
                (6.3+nf_pool(9)))*((x(4)/(14+
                x(4)));
    V_Enzyme(13)= Vmax(15)*((x(7)/(41+x(7)))*
                (0.23+(0.8*exp(-0.026*x(8))));
    V_Enzyme(14)= Vmax(16)*(((x(7)^1.21)/(300+
                (x(7)^1.21)))*(1+((7.2*(x(8)^2))/
                ((360^2)+(x(8)^2))));
    V_Enzyme(15)= Vmax(17)*((x(8)/(63+x(8)))
                *(1/(1+(x(9)/10.8)))*(4.38/
                (0.35+x(1)));
    V_Enzyme(16)= Vmax(18)*((x(10)/(1000+x(10))))*
                (((1.2*x(8))+(x(9)^2))/((30^2)+
                (x(8)+x(9))^2));
    V_Enzyme(17)= Vmax(19)*((x(8))/(1.4*(1+(x(9)/
                1.4)+x(8))));
    V_Enzyme(18)= exp(-0.0021*(x(8)+x(9))*(0.0021*
                77.2))*Vmax(20)*((x(10)*nf_pool(6))
                /((12+x(10))*(100+nf_pool(6))));
    V_Enzyme(19)= Vmax(21)*(x(9)/(10+x(9)))-Vmax(22)
                *(x(10)/(1+x(10)));

    V_Enzyme;
```

A4: Combo_Effect Annotated Matlab Program

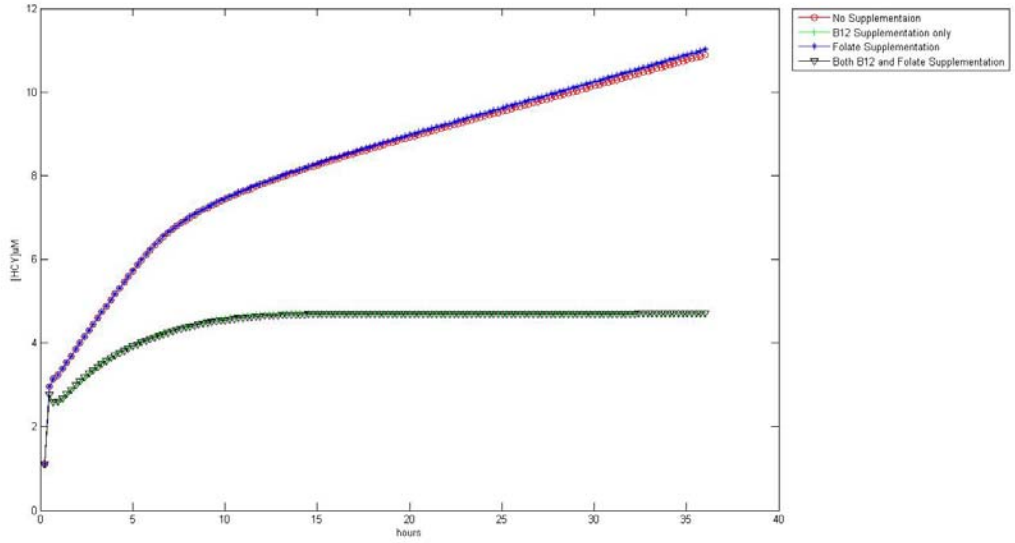
```
1   function combo_effect = Combo(z)
2   %This function serves two purposes, to generate all
3   %of the SNP combinations and to assign the
4   %respective modifiers
5
6   global s;
7   %Line 6 refers to the global variable, s, that
8   %is originally defined in the %“ODE” function.
9
10  n=s;
11  %Line 10 declares and defines a variable, n, to
12  %equal whatever s is atthe time. So first run
13  %through, s=n=1. Second run through, s=n=2, etc.
14
15  MTHFR = [1 2 3 4 5 6];
16  %Line 15 refers to the C667T polymorphism.
17  %Number 1 = Homozygous dominant CC,
18  %2 =Heterozygous CT, 3 = Homozygous recessive TT.
19  %4-6 refer to the A1298C
20  %polymorphism. Number 4 = Homozygous dominant AA,
21  %5 =Heterozygous AC, 6 = %Homozygous recessive CC.
22
23  MTD = [7 8 9];
24  %Number 1 = Homozygous dominant GG,
25  %2 =Heterozygous GA,  3 = Homozygous recessive AA.
26
27  MS = [10 11 12];
28  %Number 1 = Homozygous dominant AA,
29  %2 =Heterozygous AG, 3 = Homozygous recessive GG.
30
31  SHMT = [13 14 15];
32  %Number 1 = Homozygous dominant CC,
33  %2 =Heterozygous CT, 3 = Homozygous recessive TT.
34
35  sets = {MTHFR, MTD, MS, SHMT};
36  %Line 35 combines all of the genotypes
37  %mentioned in lines 23-33 into a single matrix
38
39  [w x y z] = ndgrid(sets{:});
40  %Line 39 uses the ndgrid command to generate
```

```
%all the possible combinations of SNPs

combo = [w(:) x(:) y(:) z(:)];
%Line 43 defines matrix "combo" to be the
%result of the line 31

selected_combo = combo (n,:);
%Line 47 selects the row vector n in the matrix
%"combo". Recall that n is equal to the global
%variable s. Currently, for every cycle, s increases
%by 1, thus for every cycle, the code will select
%the next SNP combo in the %matrix "combo". In other
%words, the code will move down to the next row in
%the matrix.

%MTHFR Polymorphisms
if selected_combo(1) == 1
        selected_combo(1) = 1;
    elseif selected_combo(1) == 2
         selected_combo(1)=0.6;
    elseif selected_combo(1)==3
         selected_combo(1)=0.3;
    elseif selected_combo(1)==4
         selected_combo(1)=1;
    elseif selected_combo(1)==5
         selected_combo(1)=0.9;
else
        selected_combo(1)=0.68;
end
%In this if-statement, the appropriate modifiers are
%applied to the corresponding SNP combination.
%In matrix "combo", the entire first column refers
%to MTHFR. For example, if a row, say [2 8 11 15],
%2 would refer to the polymorphism of MTHFR c667t.
%Specifically the heterozygousgenotype MTHFR 677 CT.
%Following the code from line 52, the program checks
%if the first element in the selected SNP combo is
%equal to 1. If element is equal to 1, then the
%value remains 1. Else, if the first element is
%equal to 2, then the first element is changed from
%2 to 0.6. This continues down the entire
%if-statement. These decimal numbers representthe
```

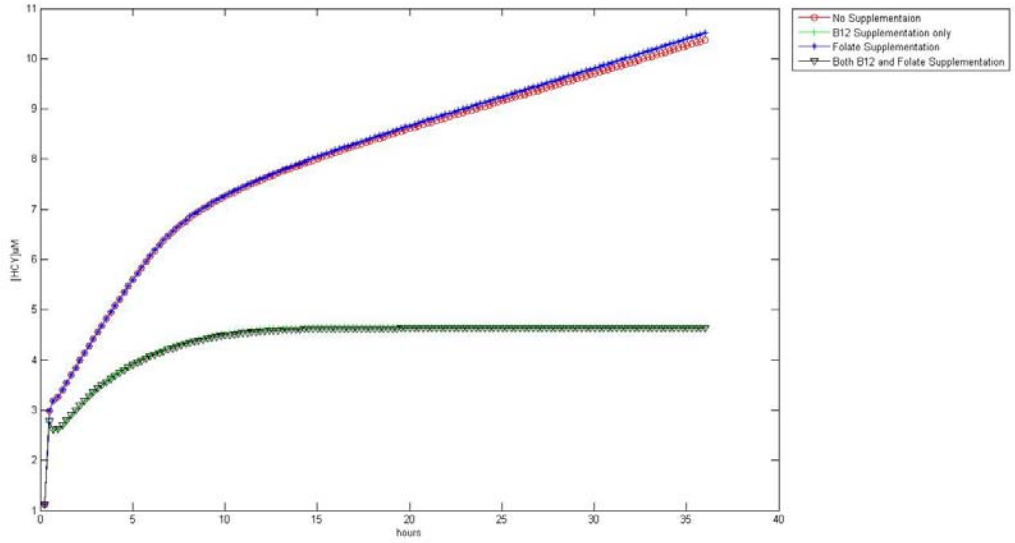
```
% percent change in activity of the corresponding
%enzyme due to thepolymorphism. In thisexample,
%the heterozygous MTHFR 677 CT polymorphism
%causes MTHFR to perform at 60% of its optimal
%efficiency (value is literature based).

%MTD Polymorphisms
if selected_combo(2) == 7
    selected_combo(2) = 1;
    elseif selected_combo(2)==8
        selected_combo(2)=0.6;
else
        selected_combo(2)=0.3;
%where selected_combo(2)==9
end


%MS Polymorphisms
if selected_combo(3) == 10
    selected_combo(3)=1;
    elseif selected_combo(3)== 11
        selected_combo(3)=0.6;
else
    selected_combo(3)=0.3;
%where selected_combo(3)==12
end

%SHMT Polymorphisms
if selected_combo(4) == 13
    selected_combo(4)=1;
    elseif selected_combo(4)== 14
        selected_combo(4)=0.6;
else
    selected_combo(4)=0.3;
%where selected_combo(4)==15
end

combo_effect=selected_combo;

%[A,B] = size(combo)
%line 122, commented out, gives the size of matrix
combo.
```

A5: Simulation Results for All Polymorphism and Nutrients

The following graphs depict the simulation results from running the homocysteine remethylation pathway model described in Chapter 2 for all 243 possible genotypes. The graphs reflect the change in homocysteine concentration over a two day period with no supplementation (red), folate only supplementation (blue), vitamin B12 only supplementation (green) and both folate and vitamin B12 supplementation (black).



MTHFR 677 CC, MTHFR 1298 AA, MTHFD1 1958 GG, MTR 2756 AA, cSHMT 1420 CC



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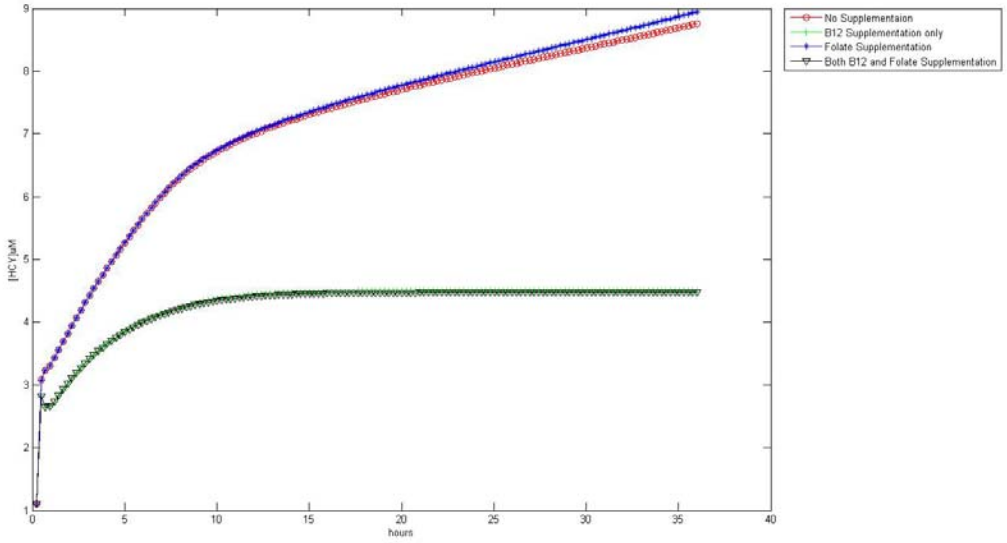

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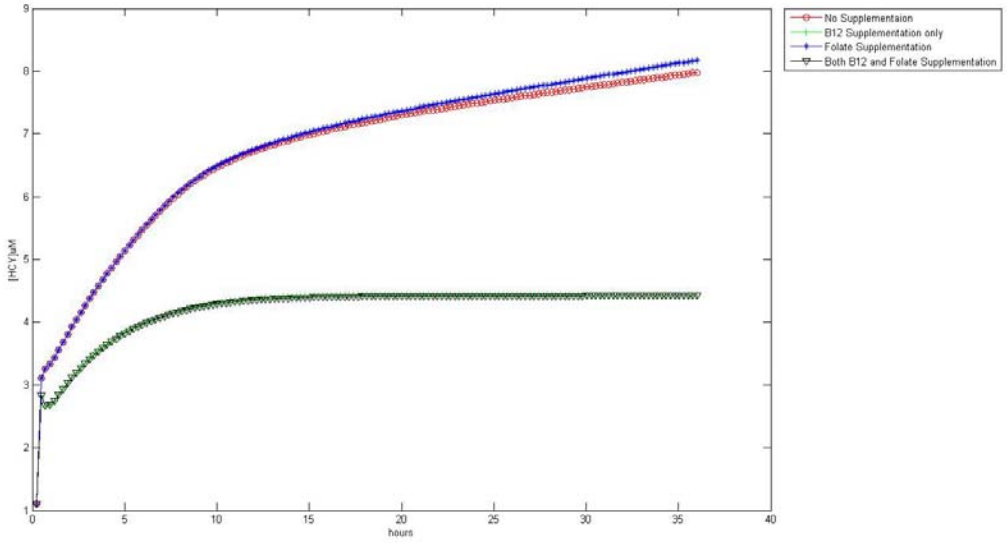

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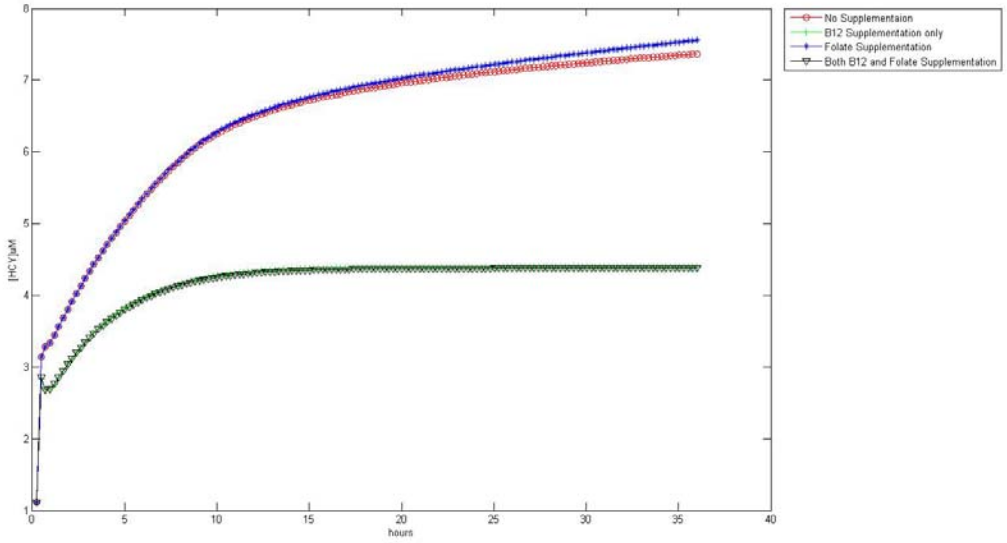

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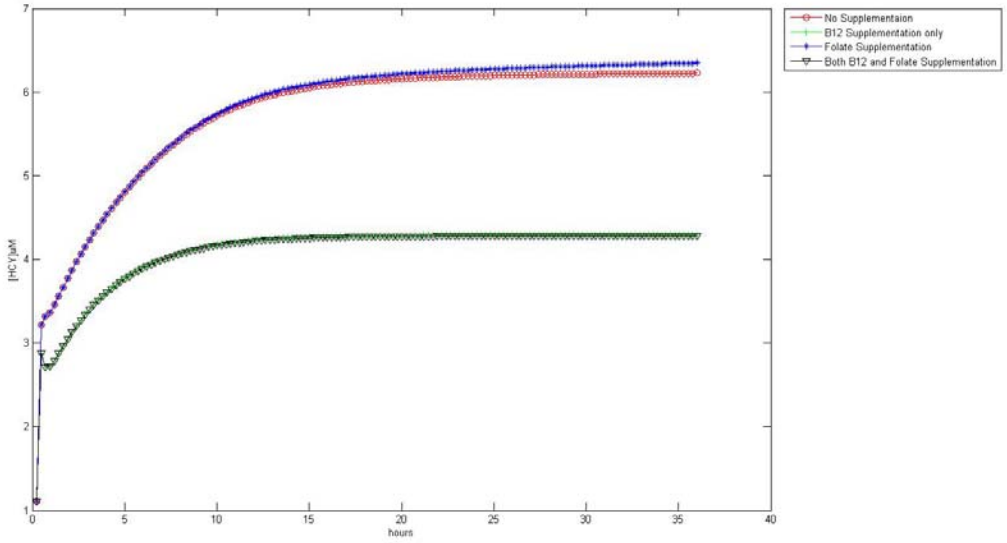

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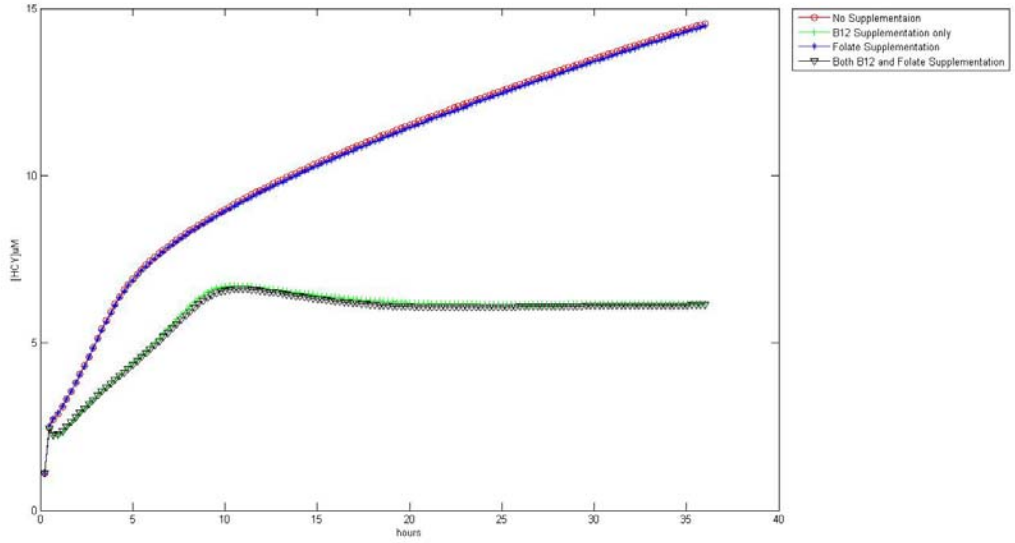

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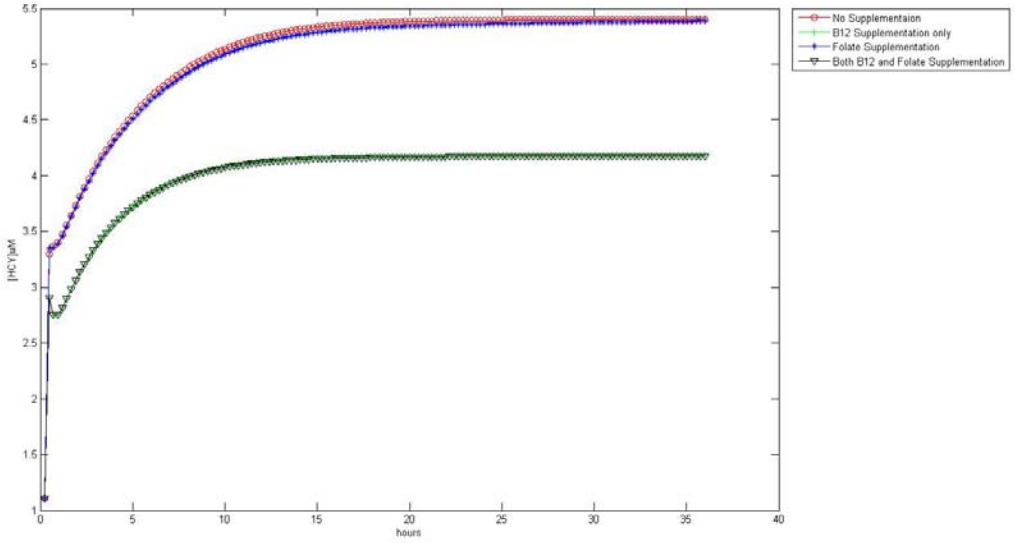

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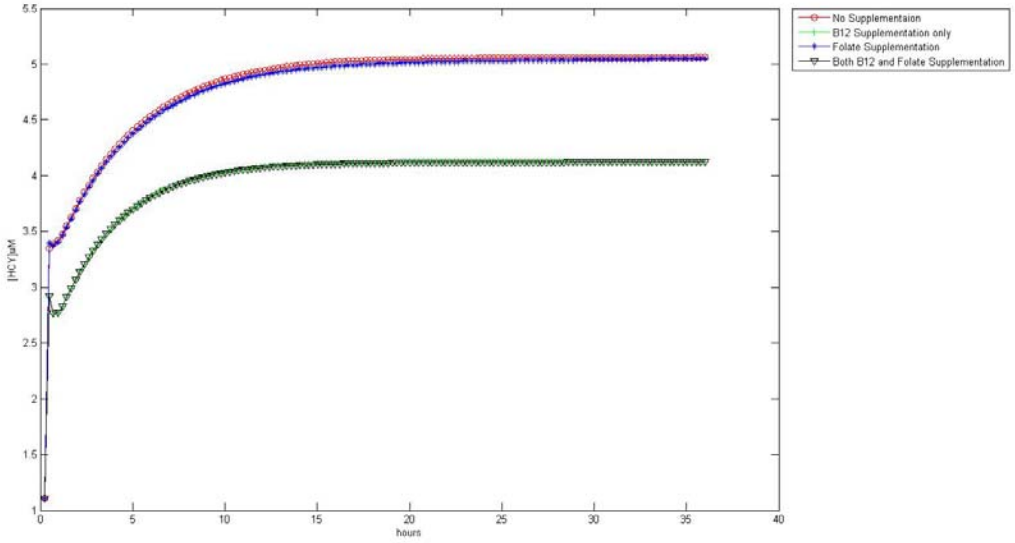

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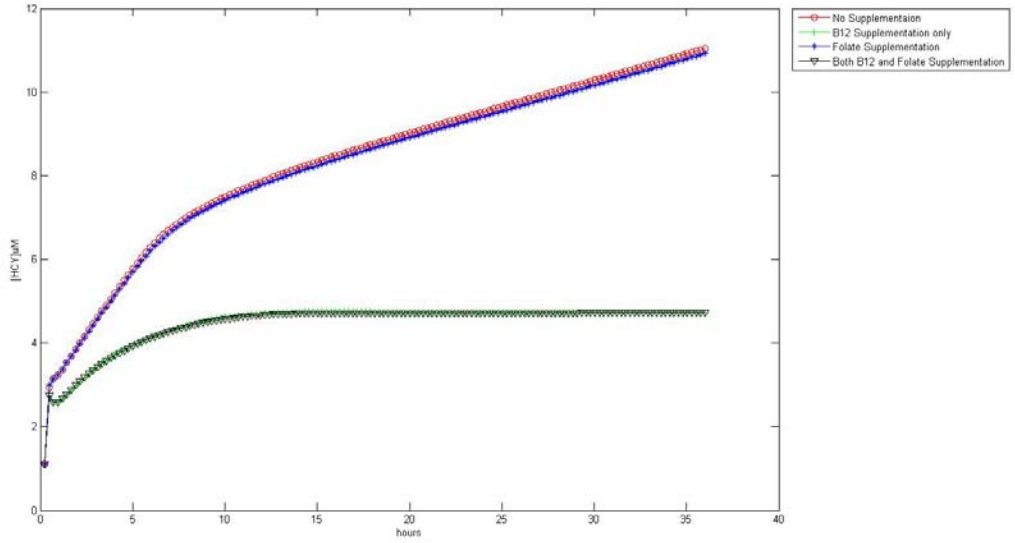

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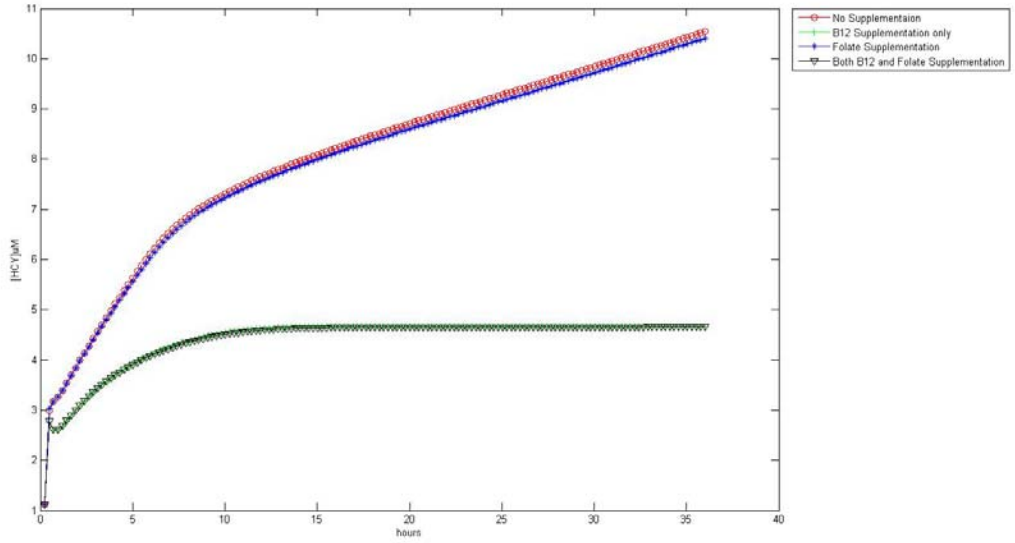

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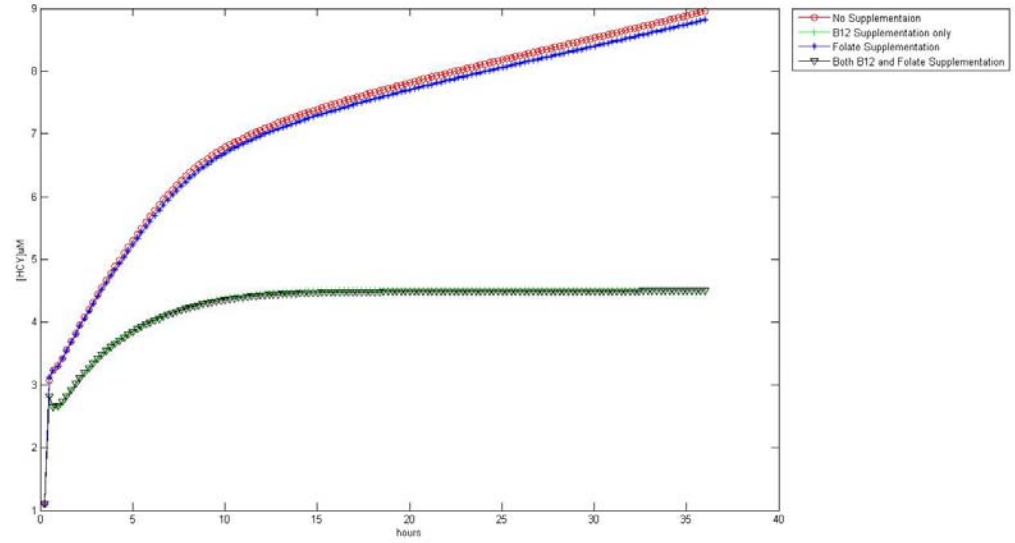

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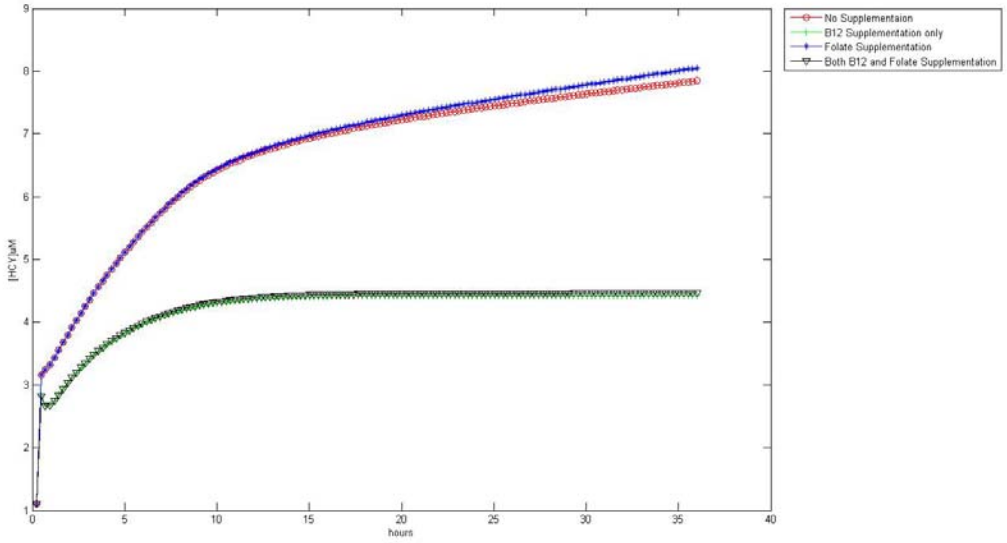

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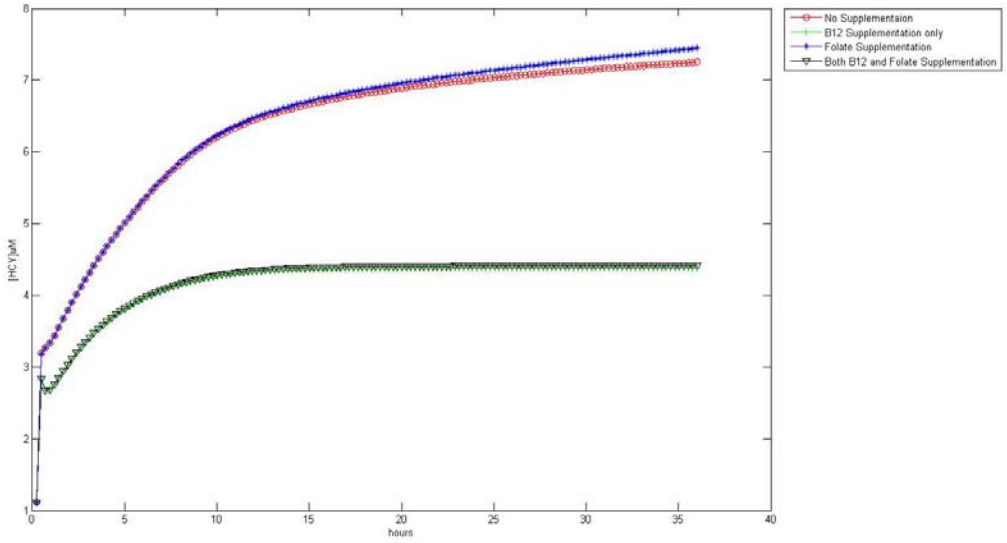

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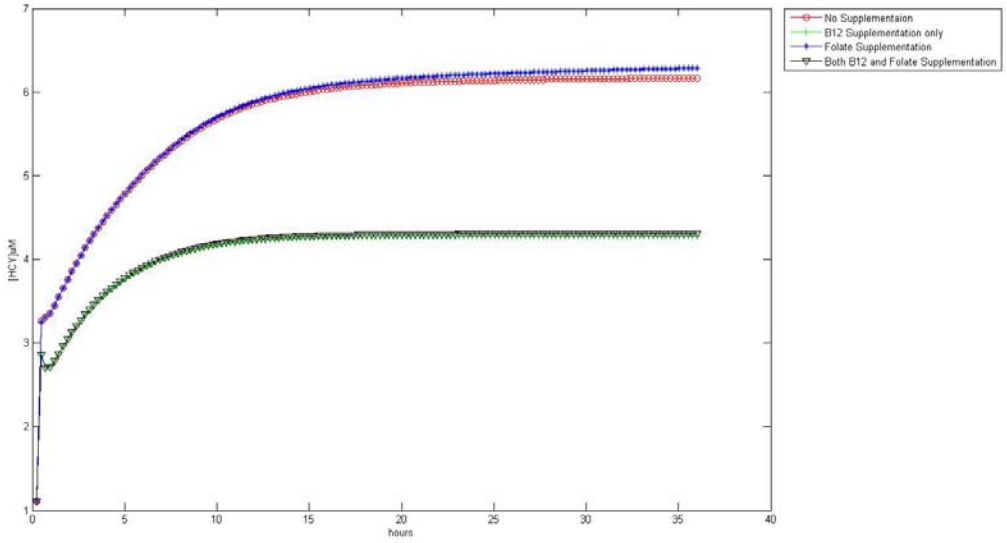

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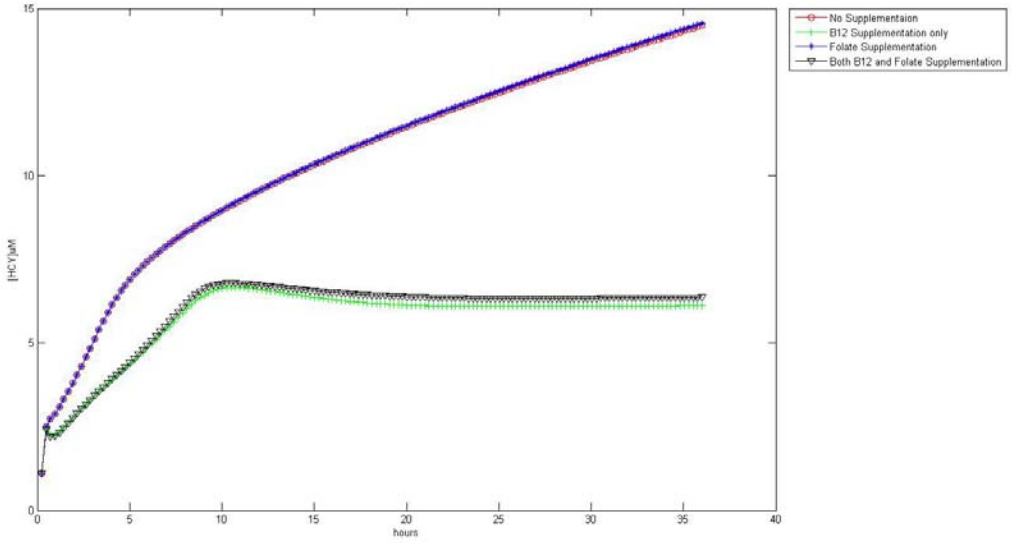

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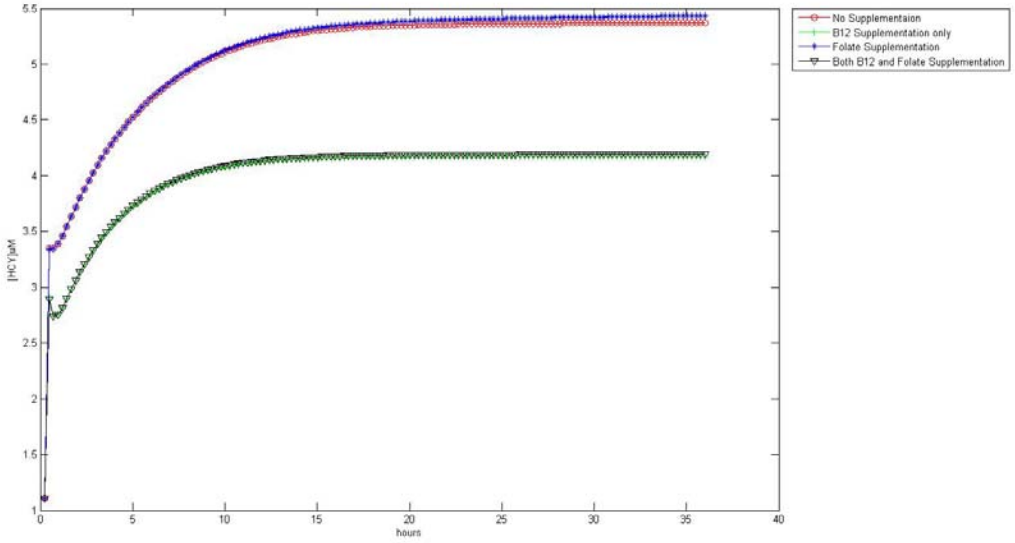

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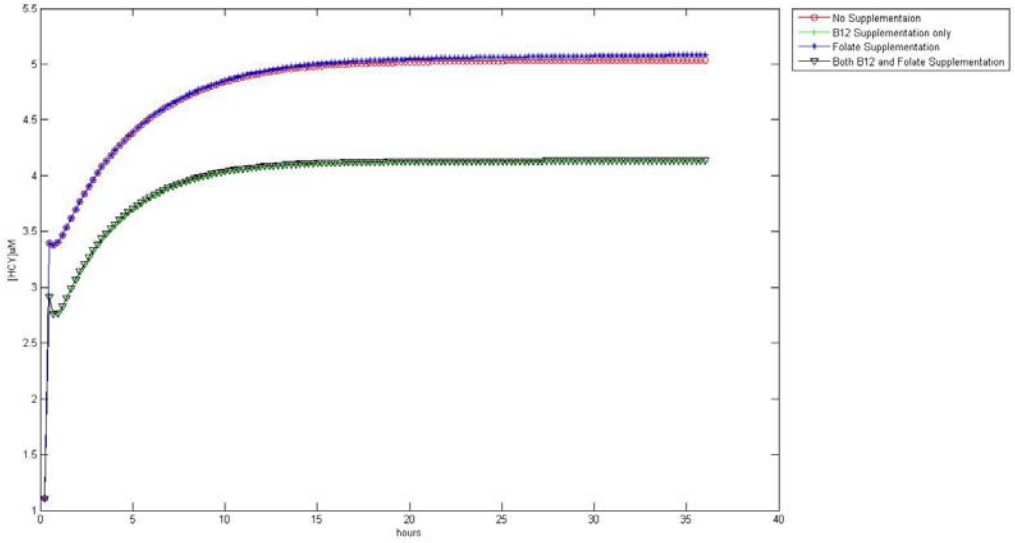

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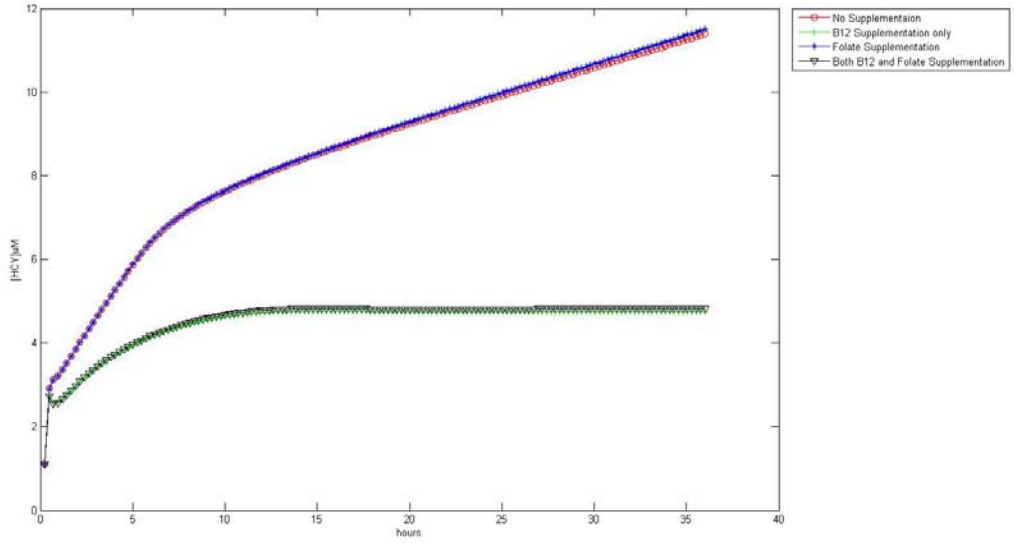

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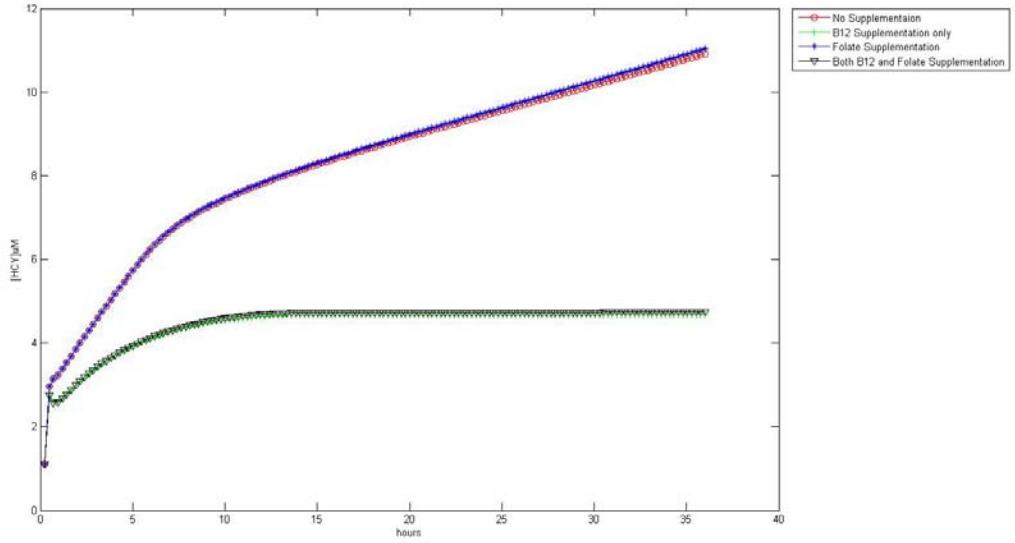

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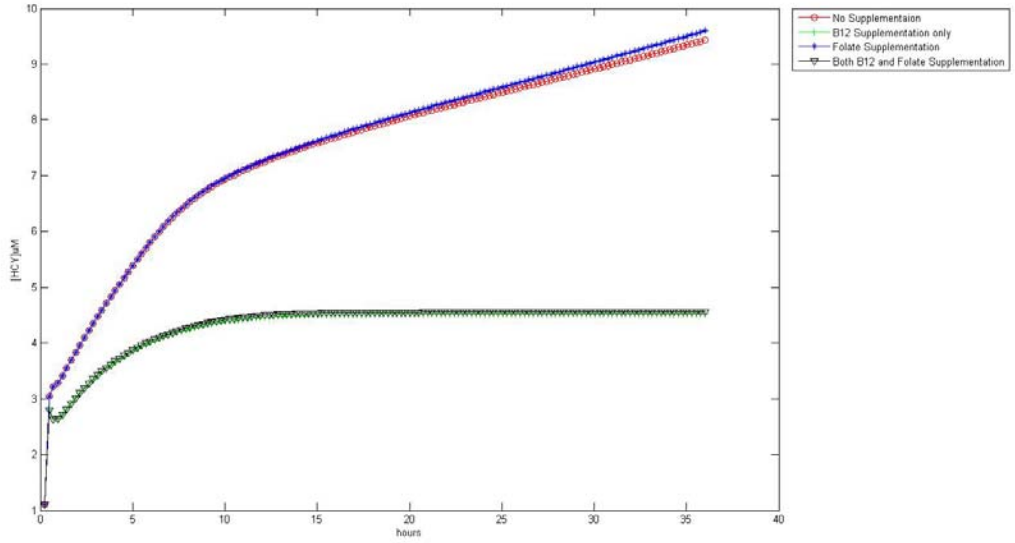

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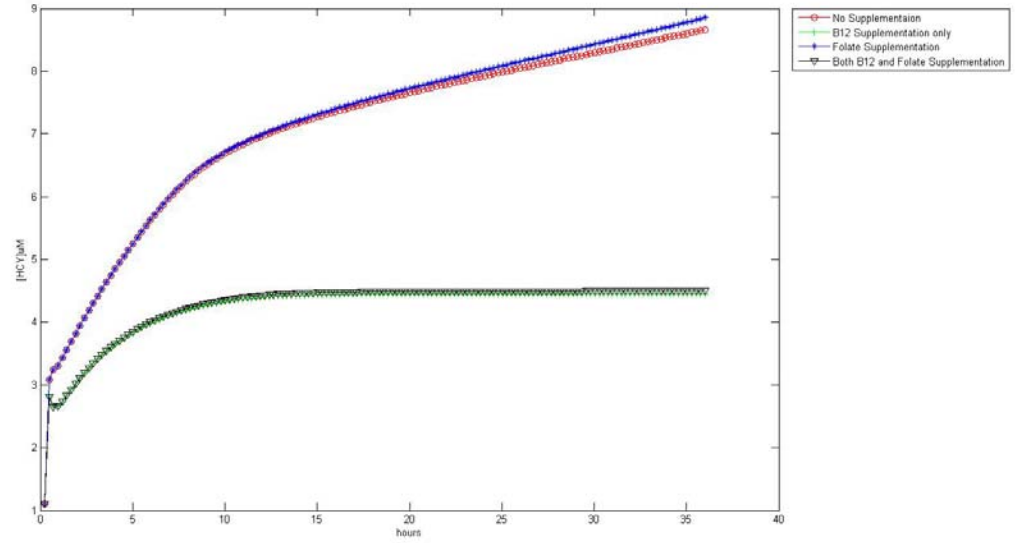

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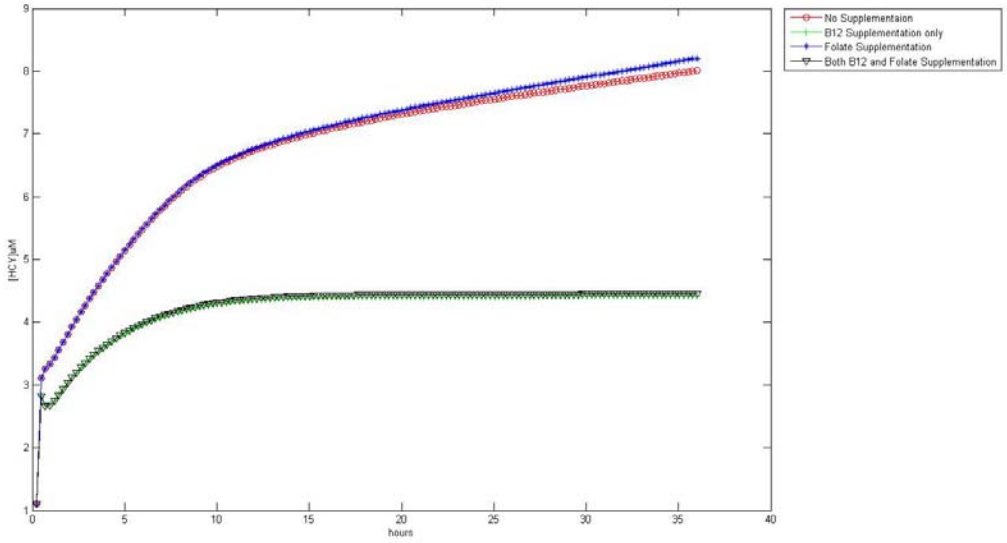

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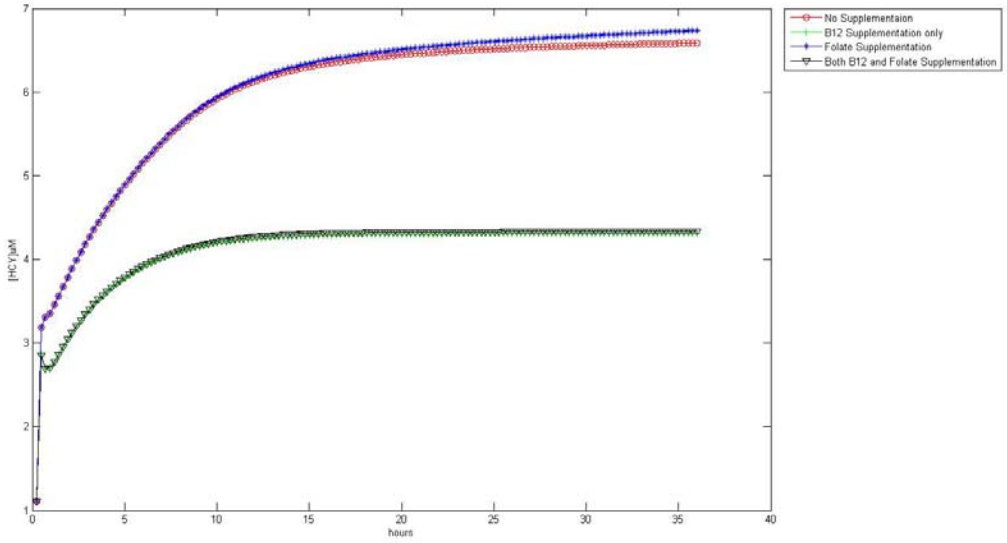

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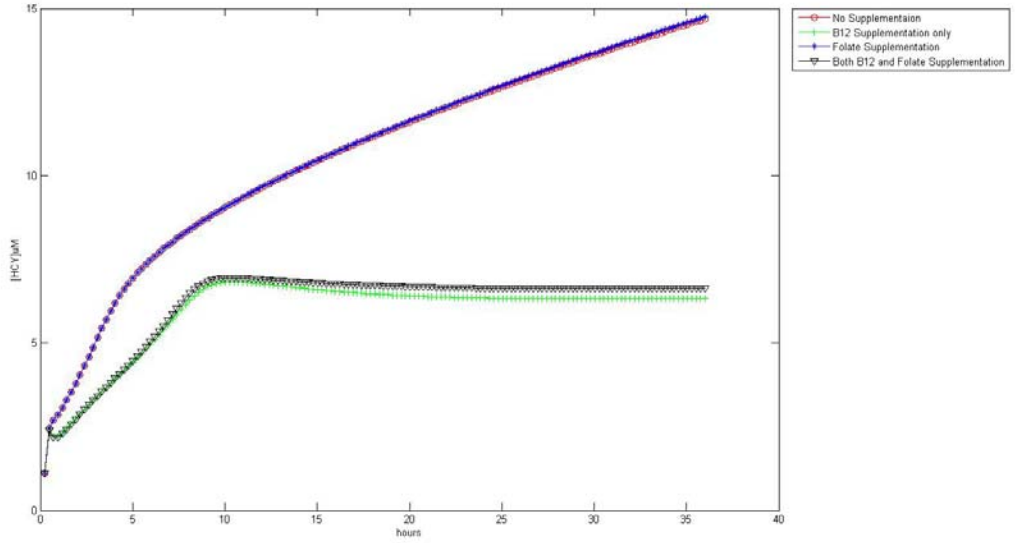

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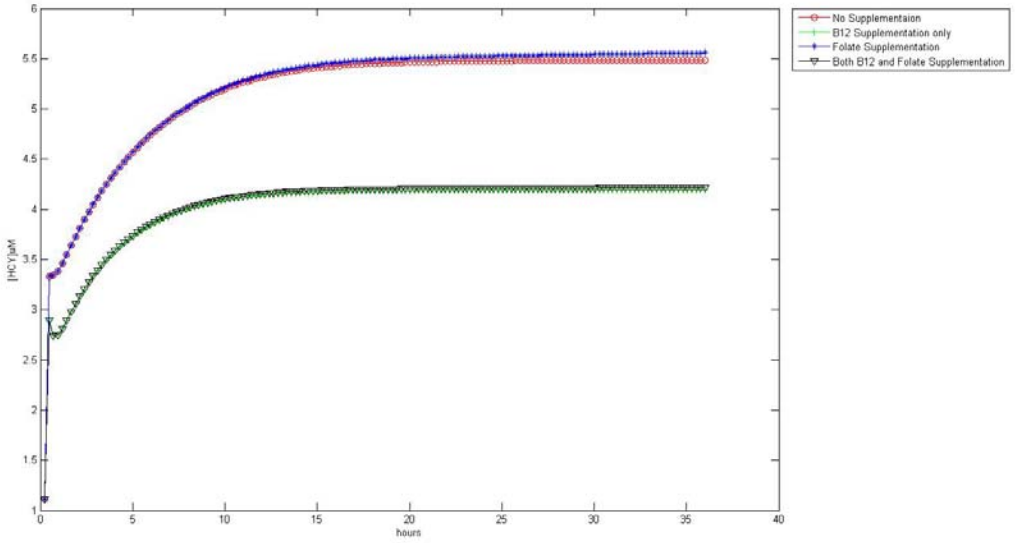

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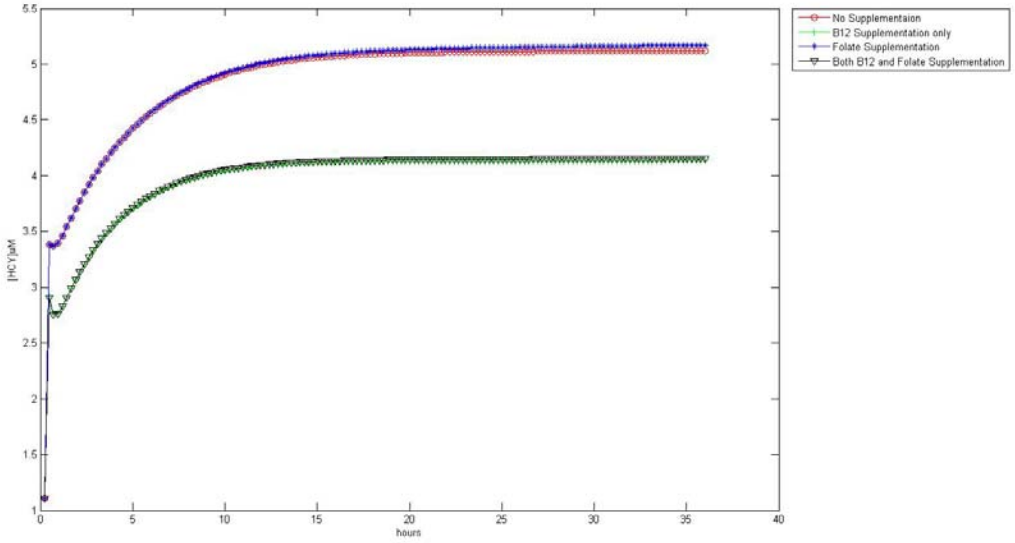

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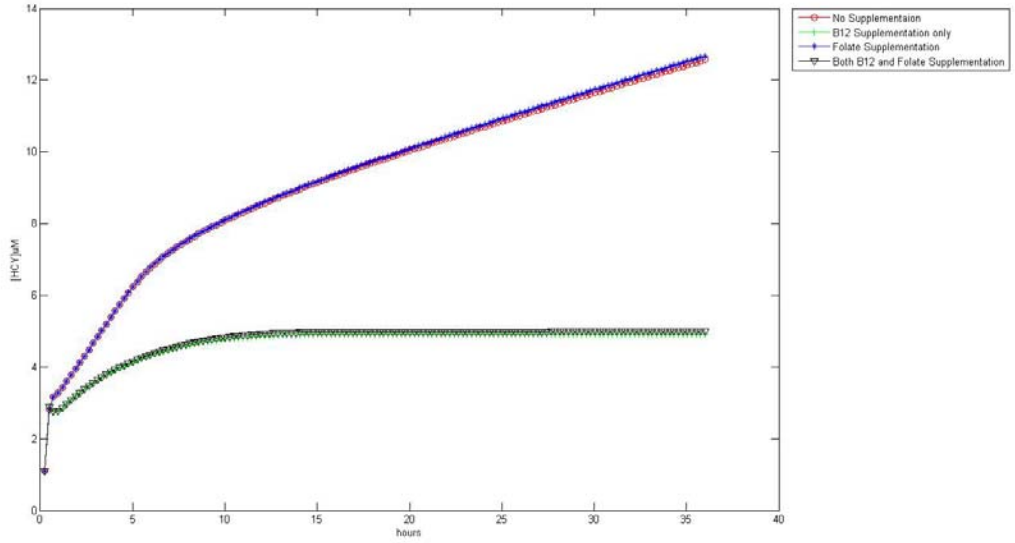

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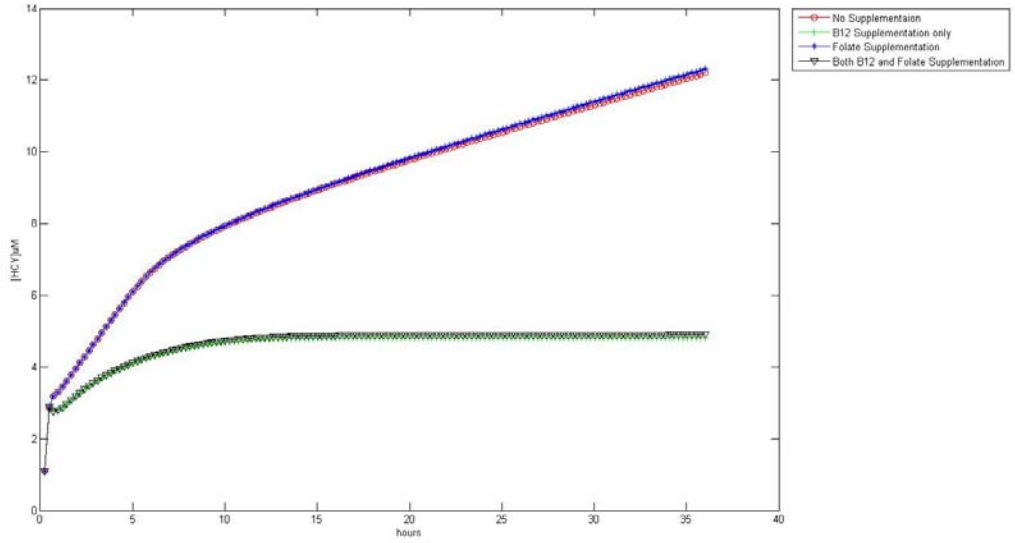

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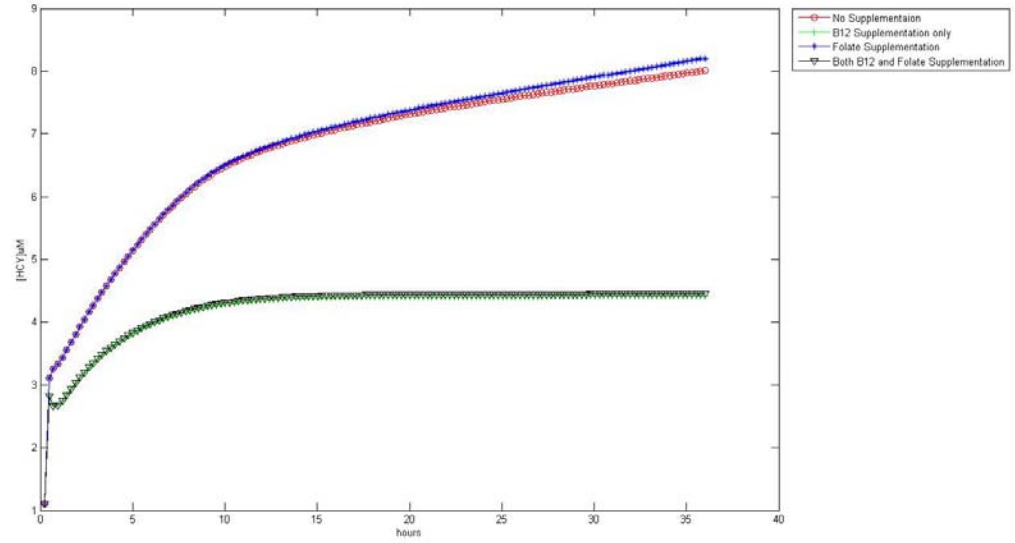

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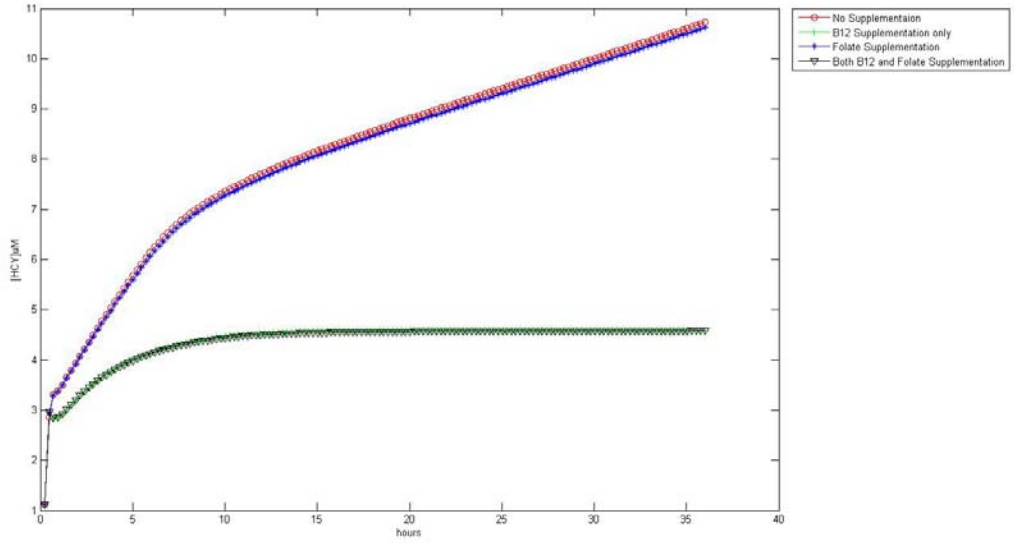

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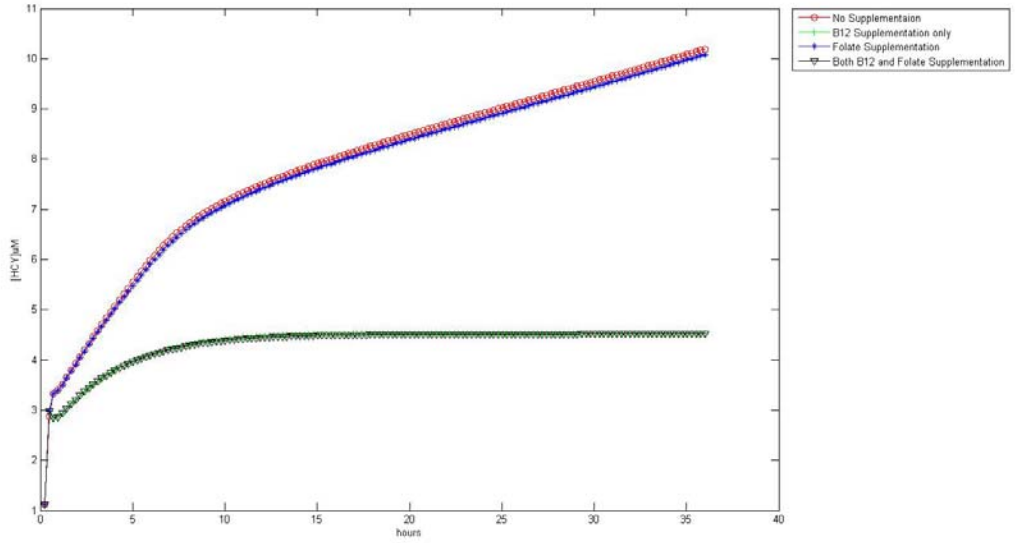

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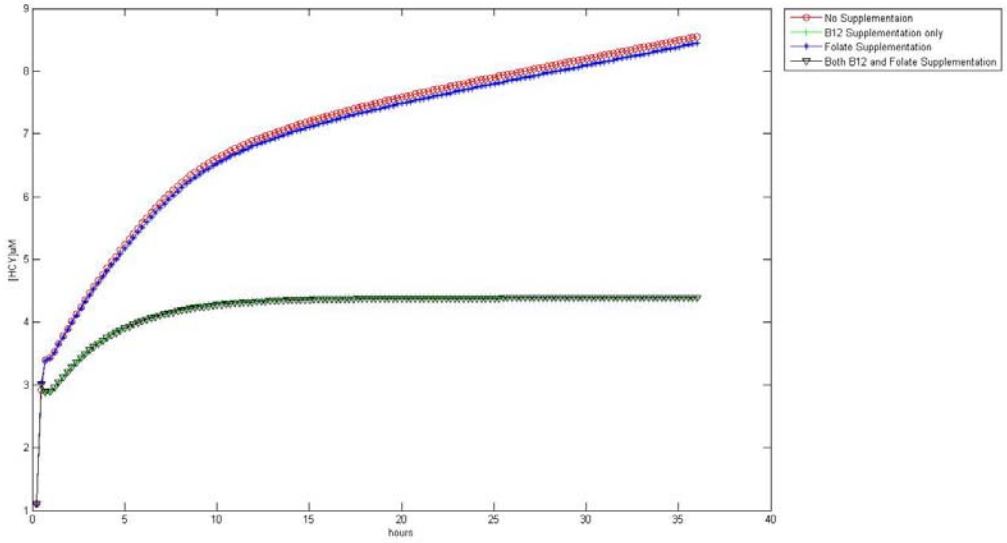

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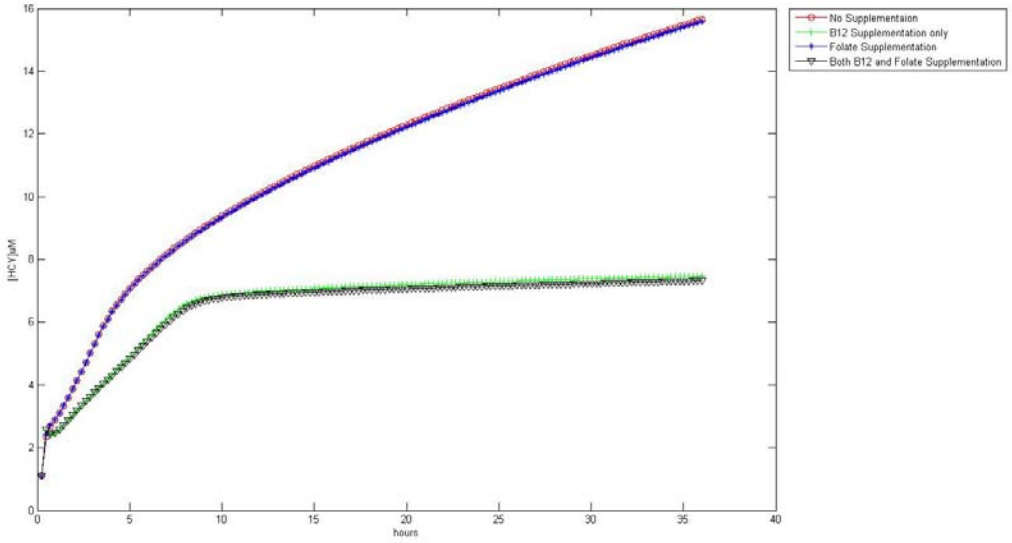

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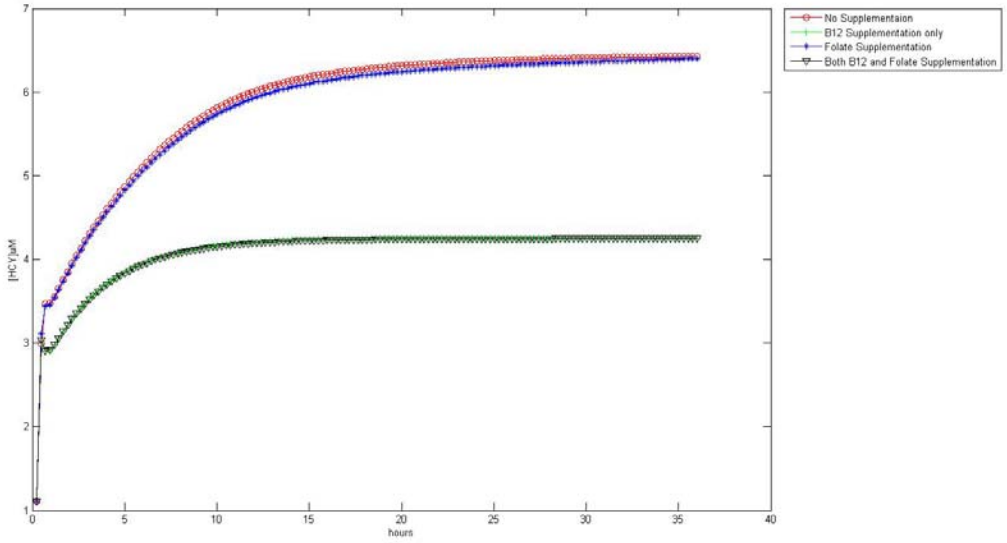

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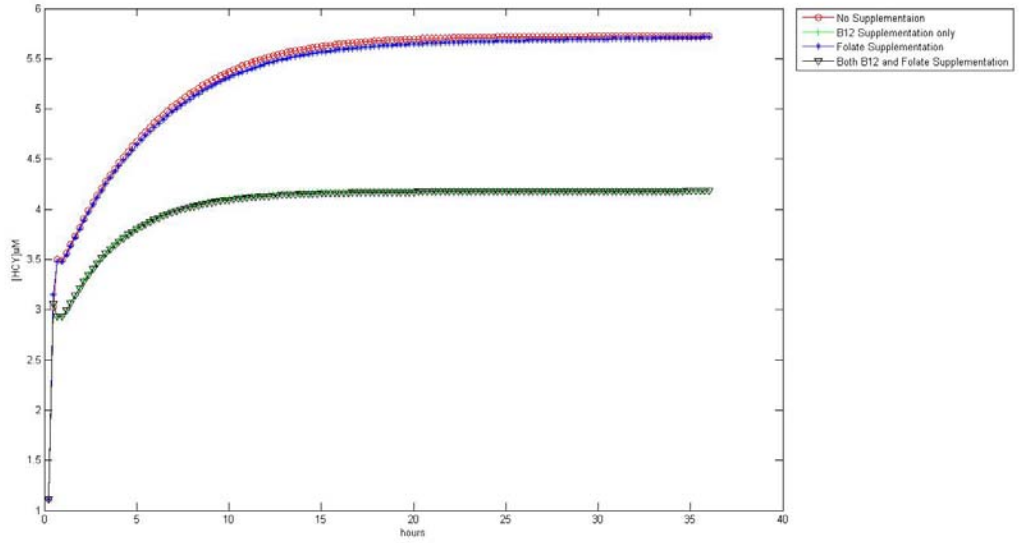

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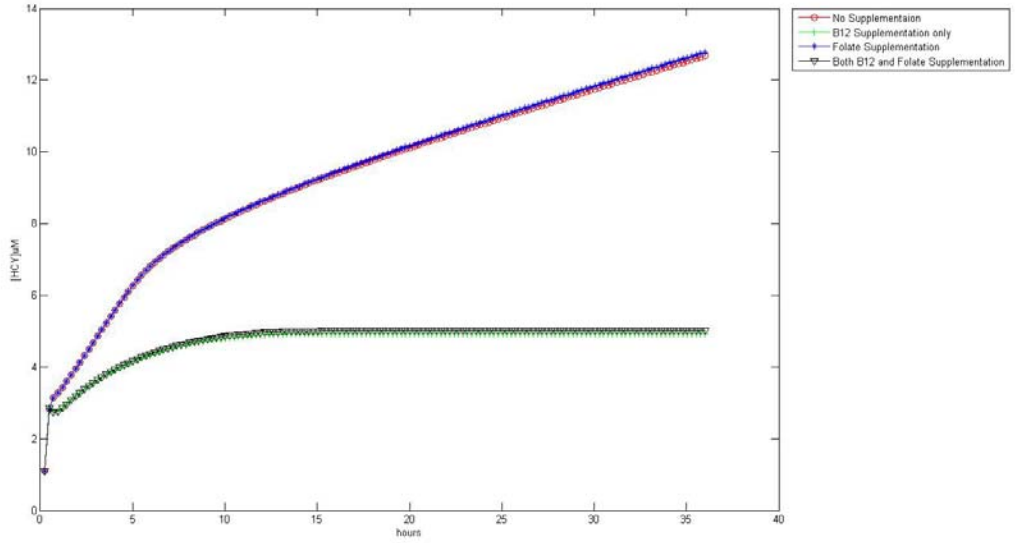

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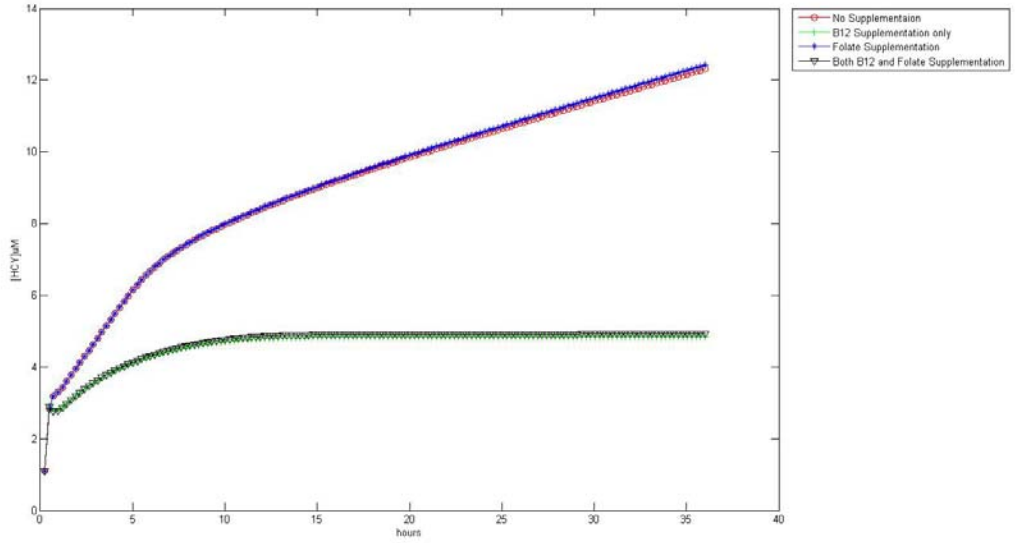

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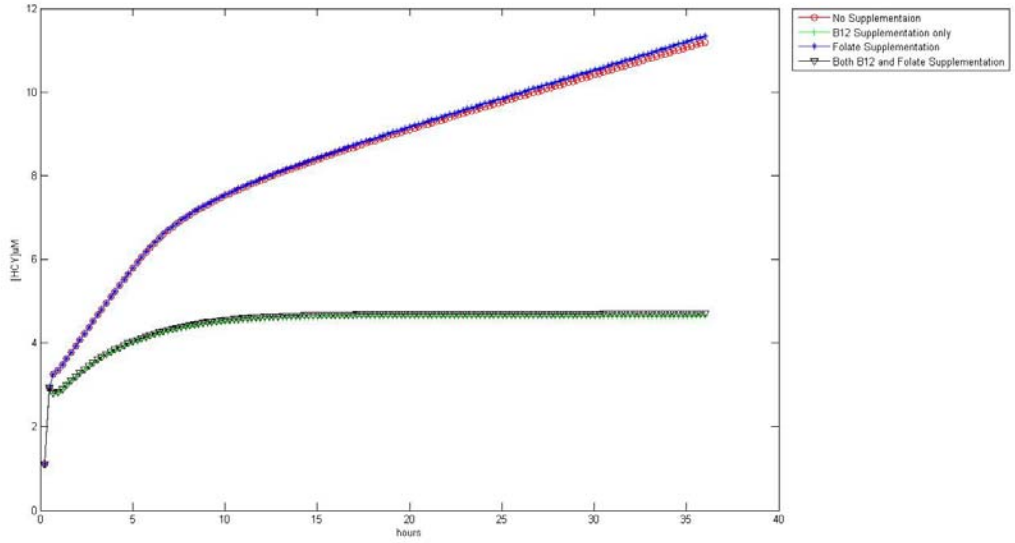

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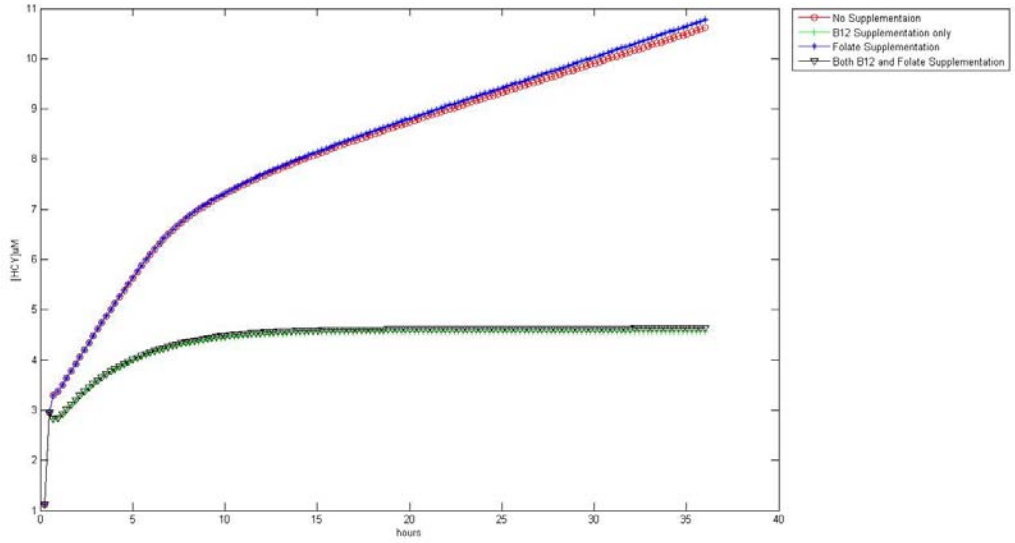

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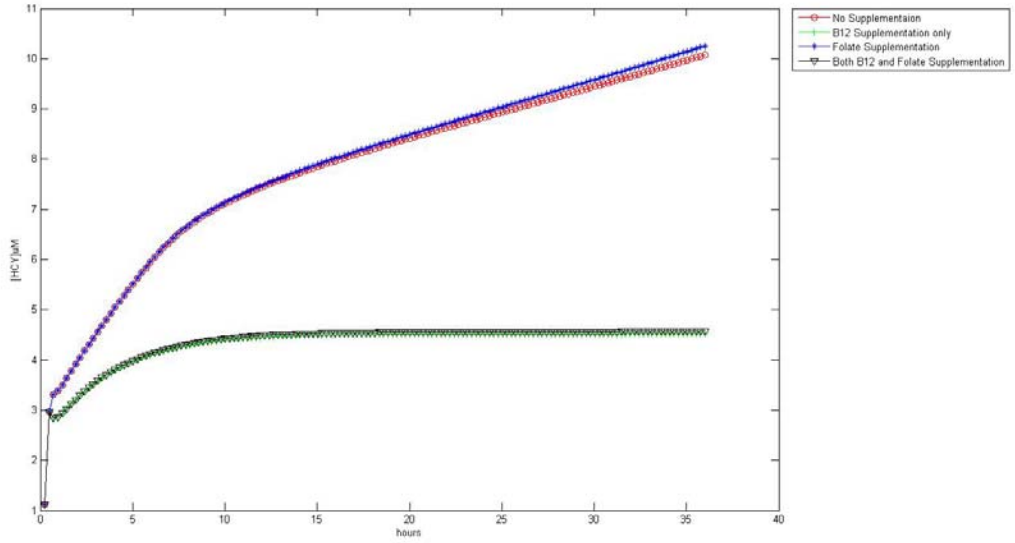

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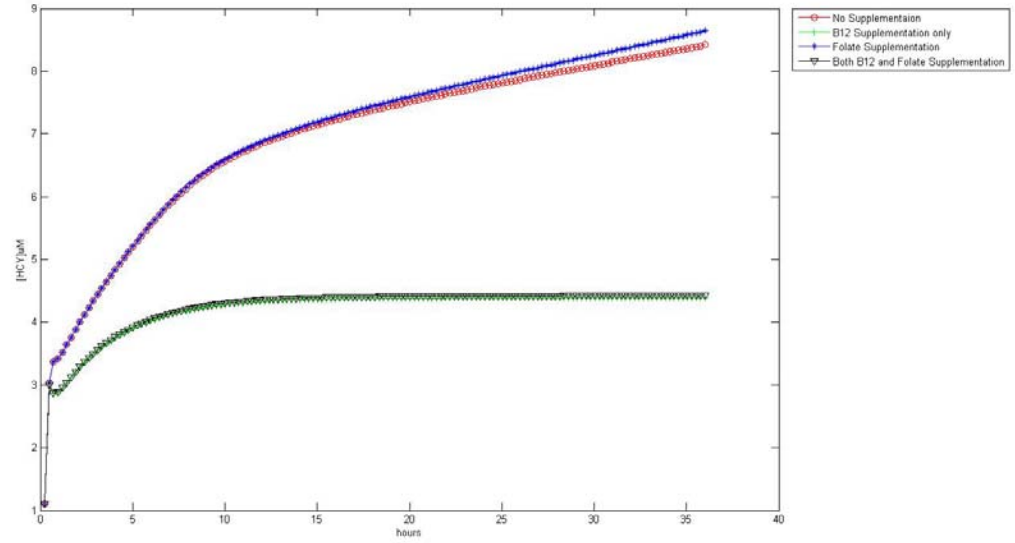

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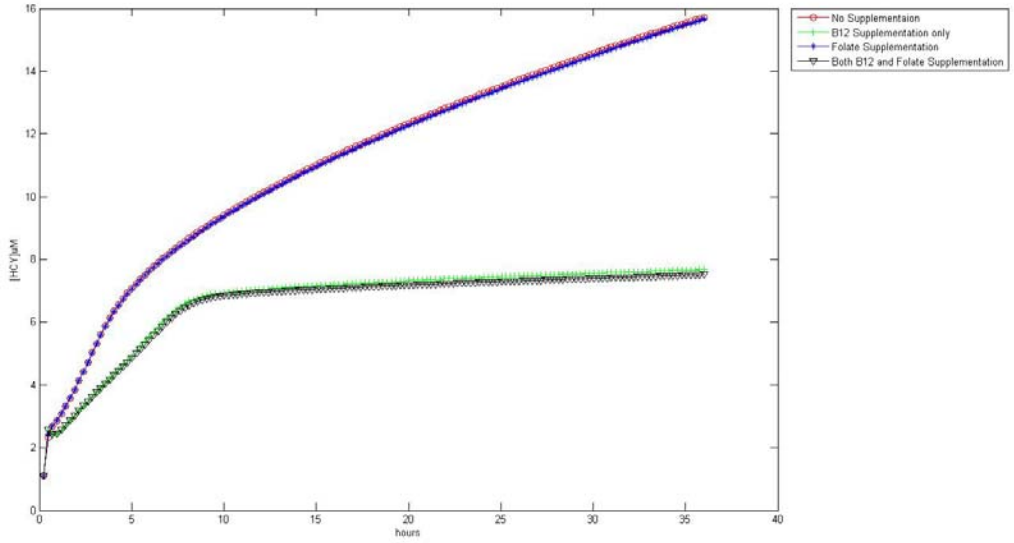

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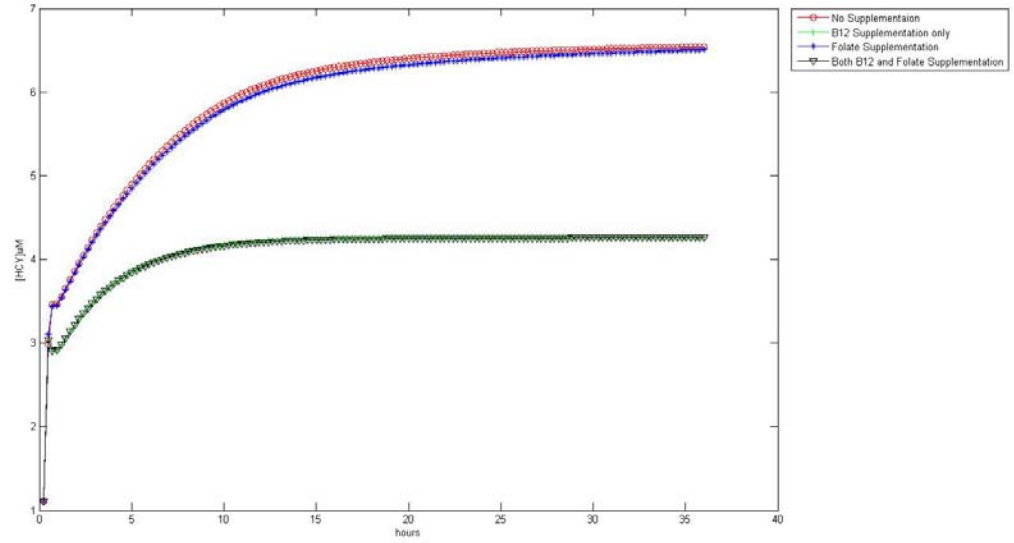

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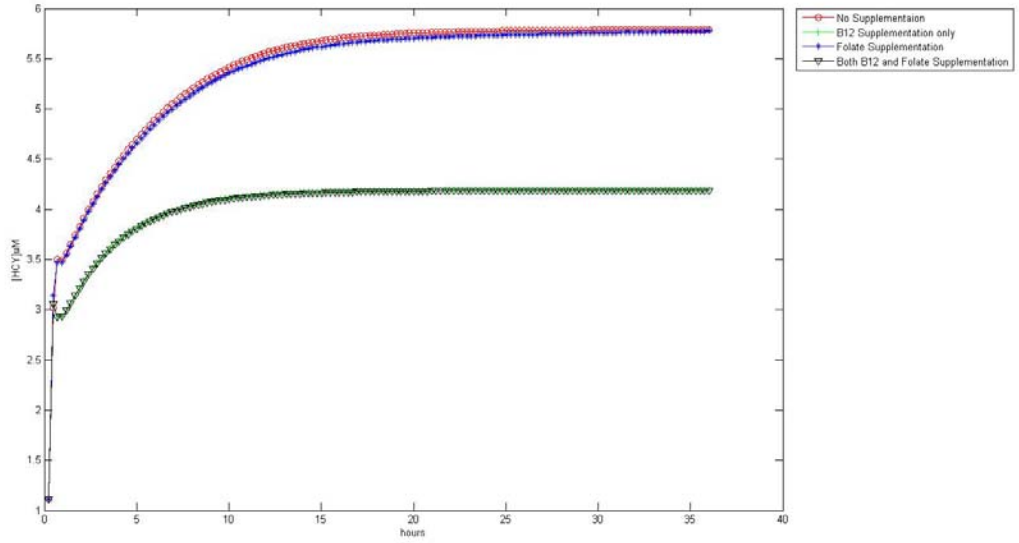

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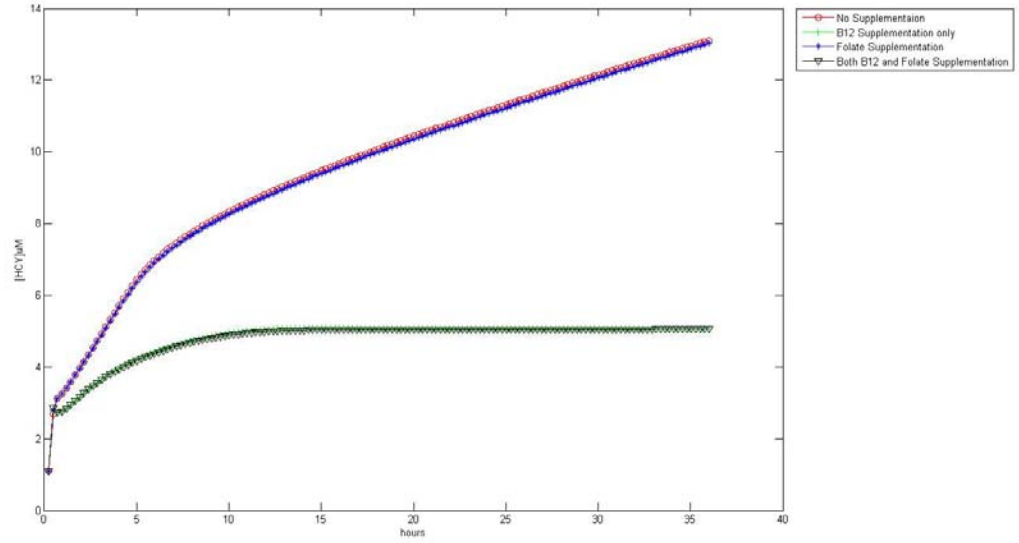

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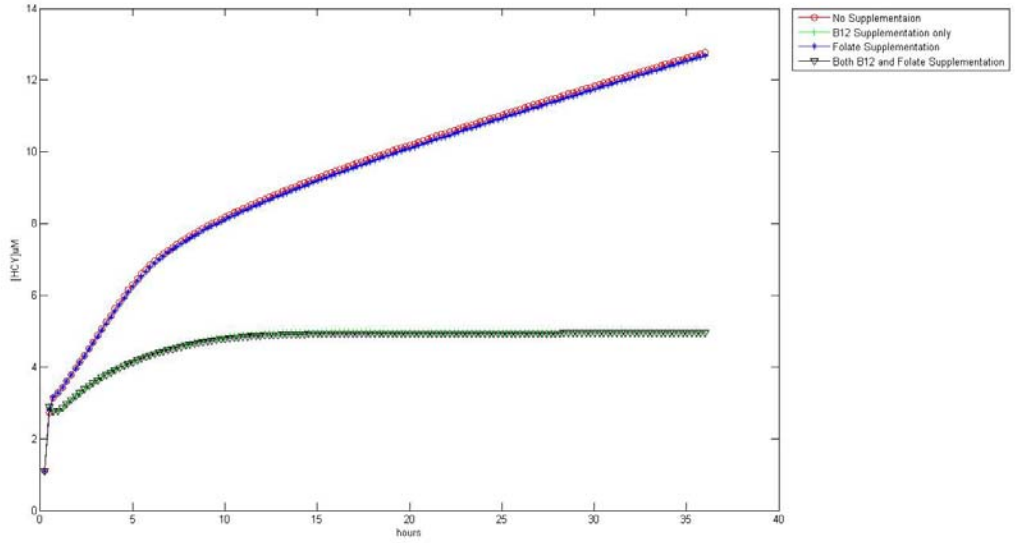

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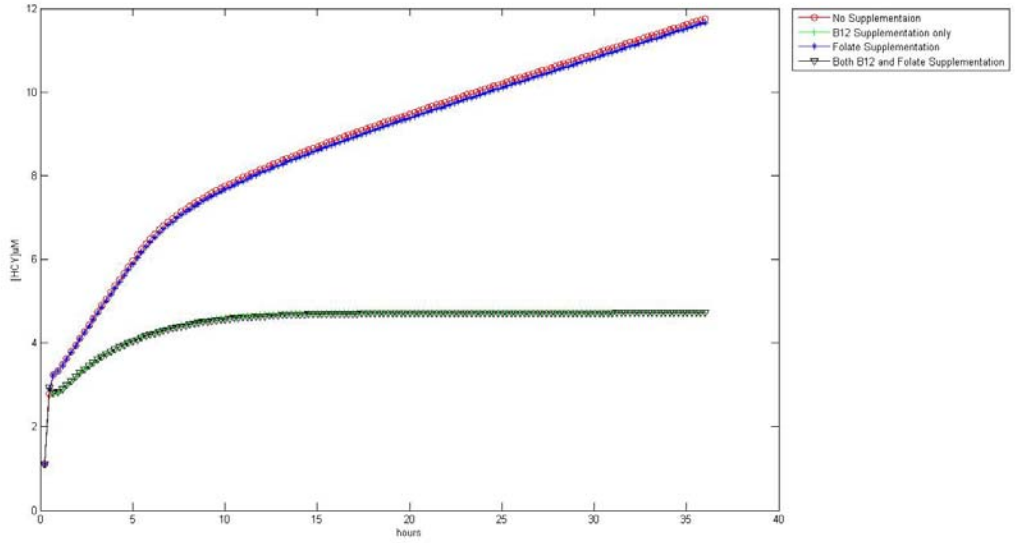

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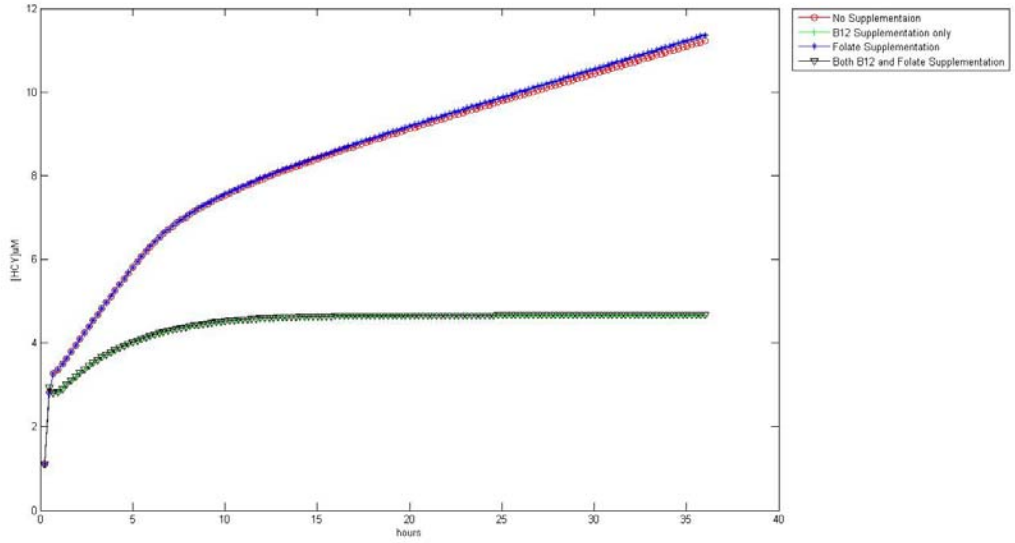

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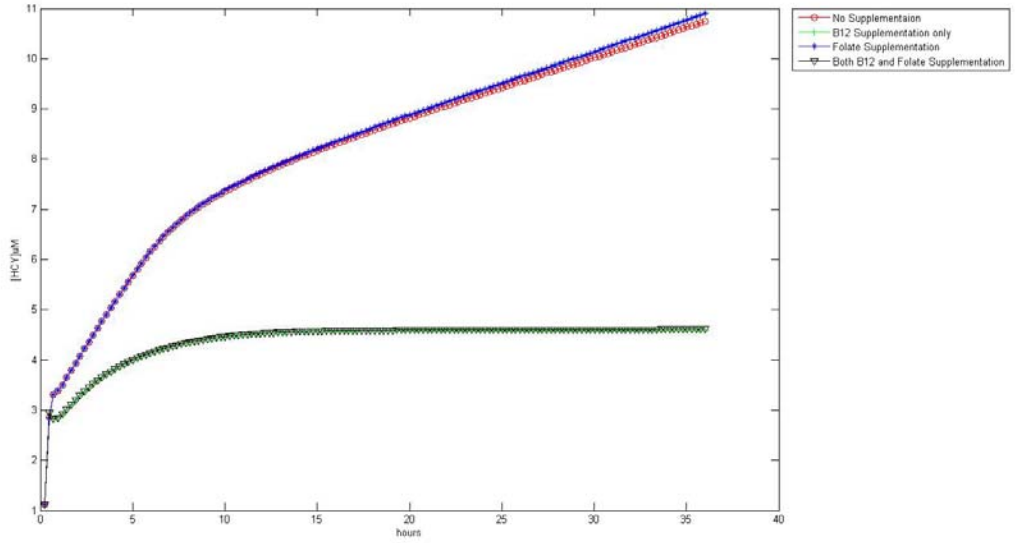

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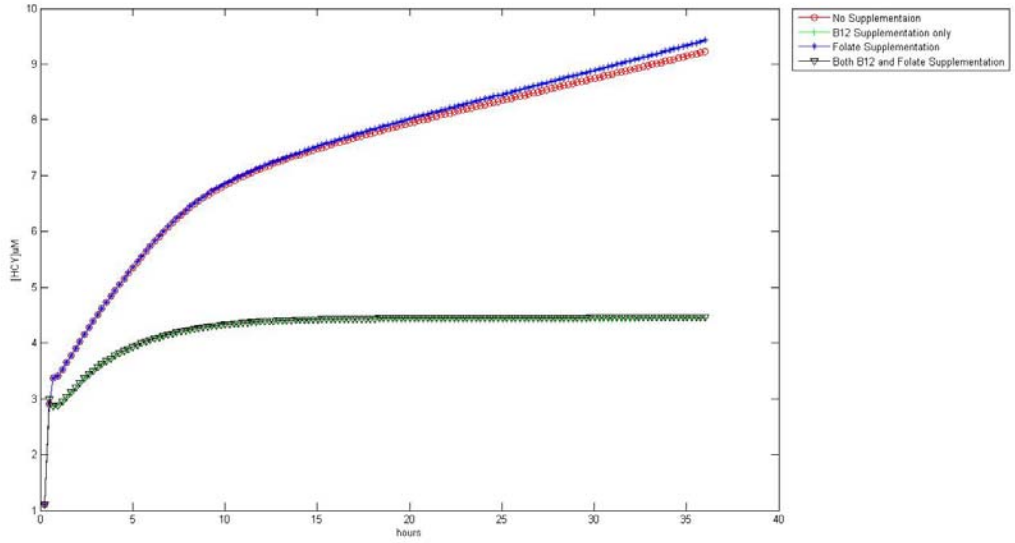

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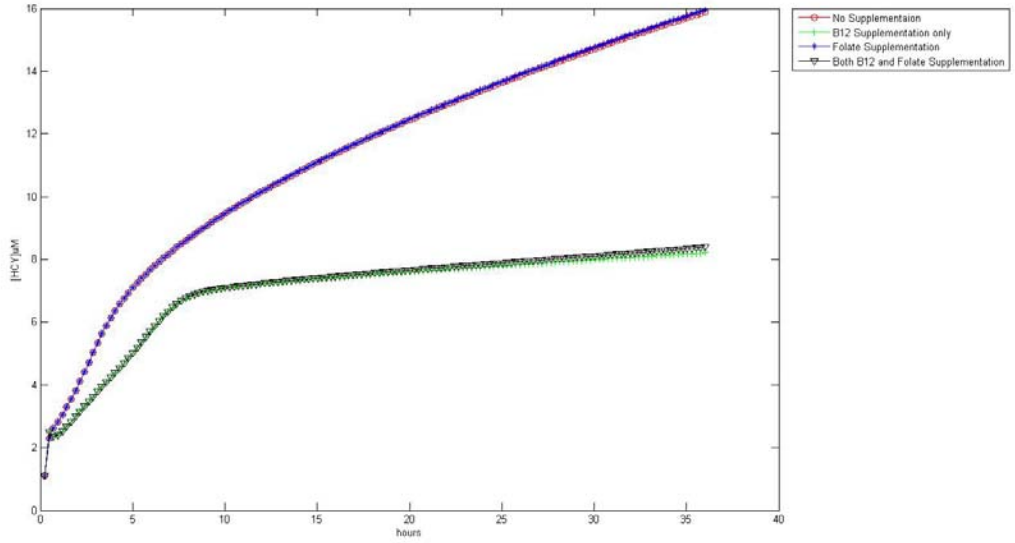

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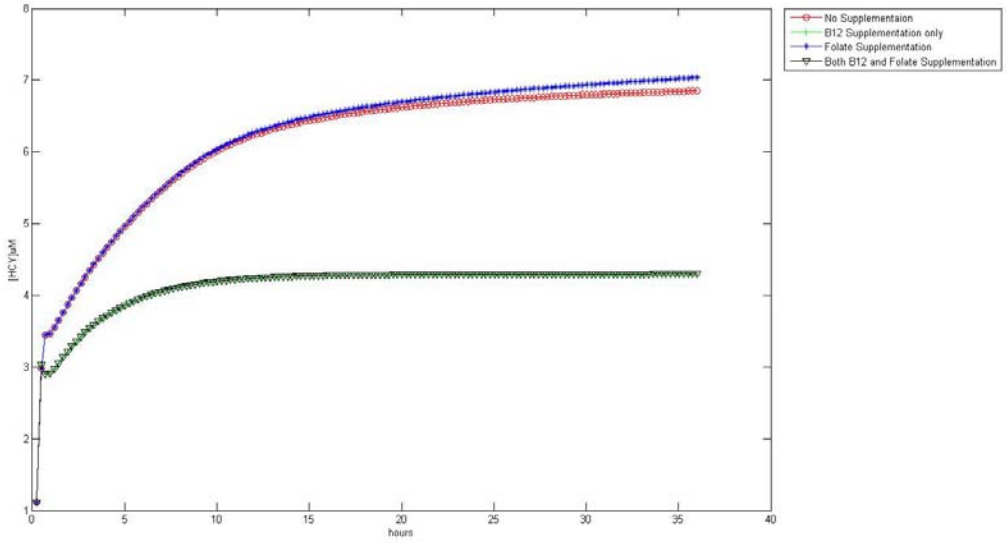

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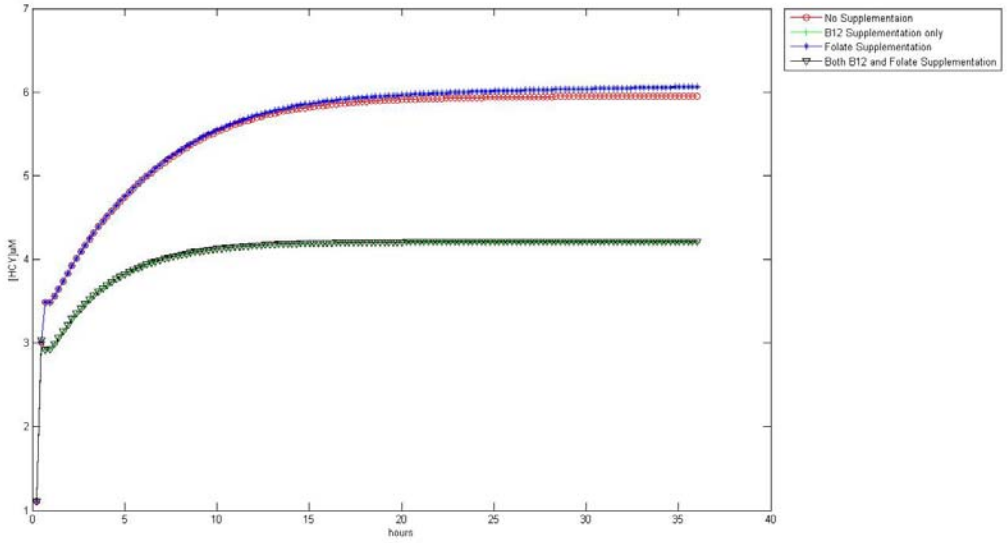

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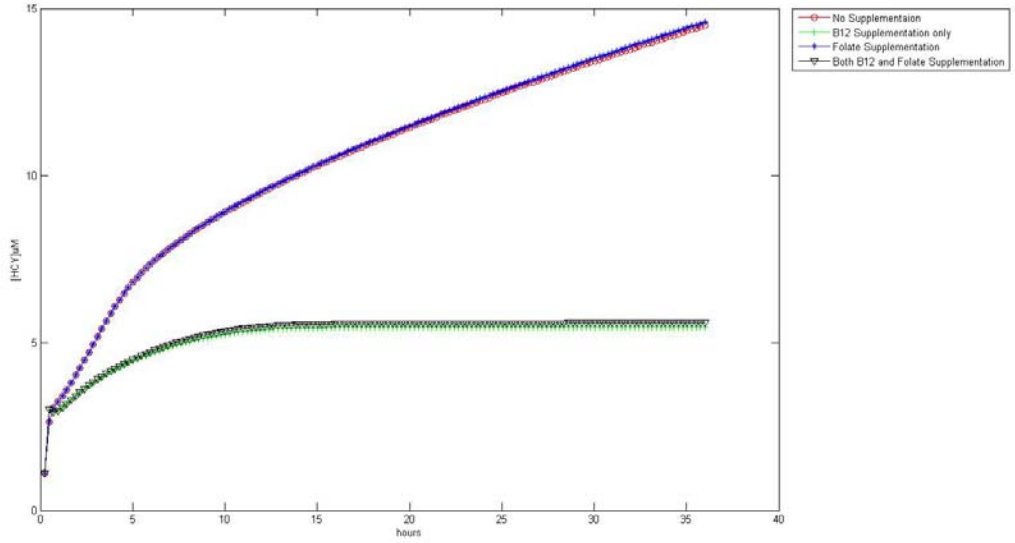

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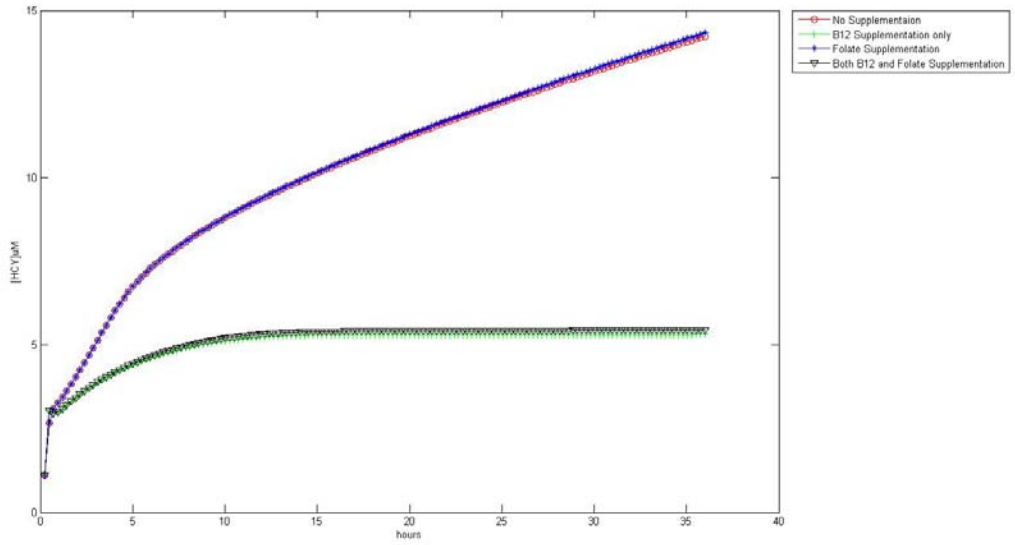

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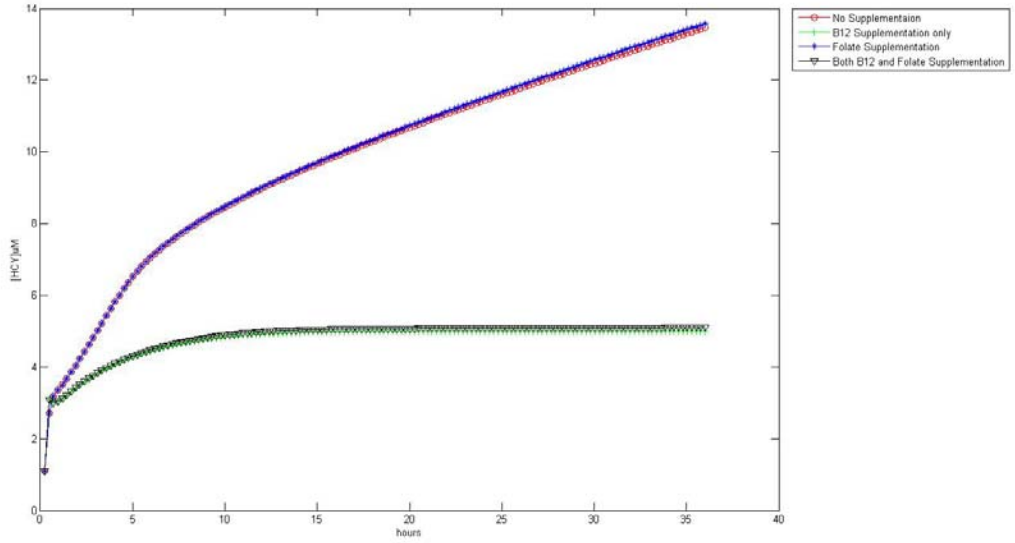

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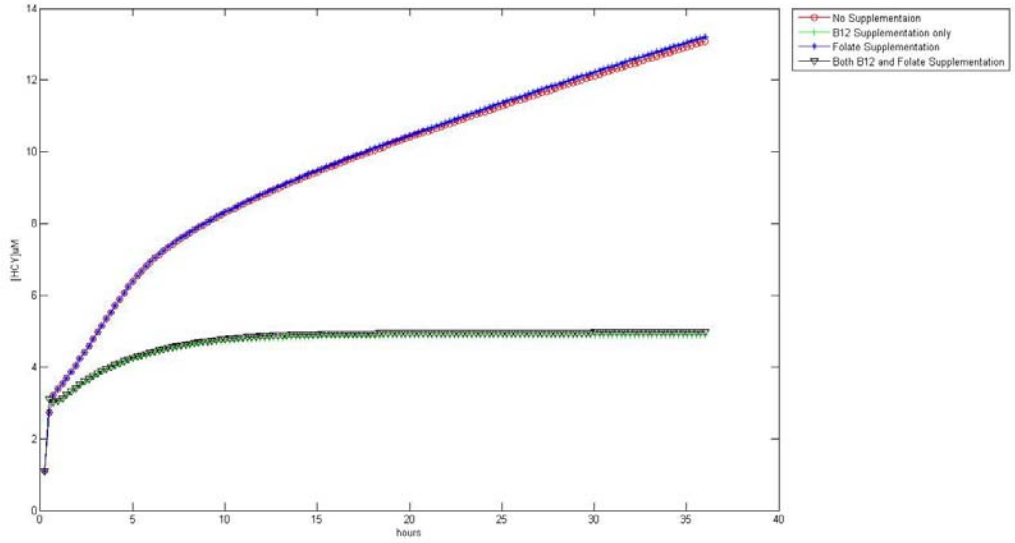

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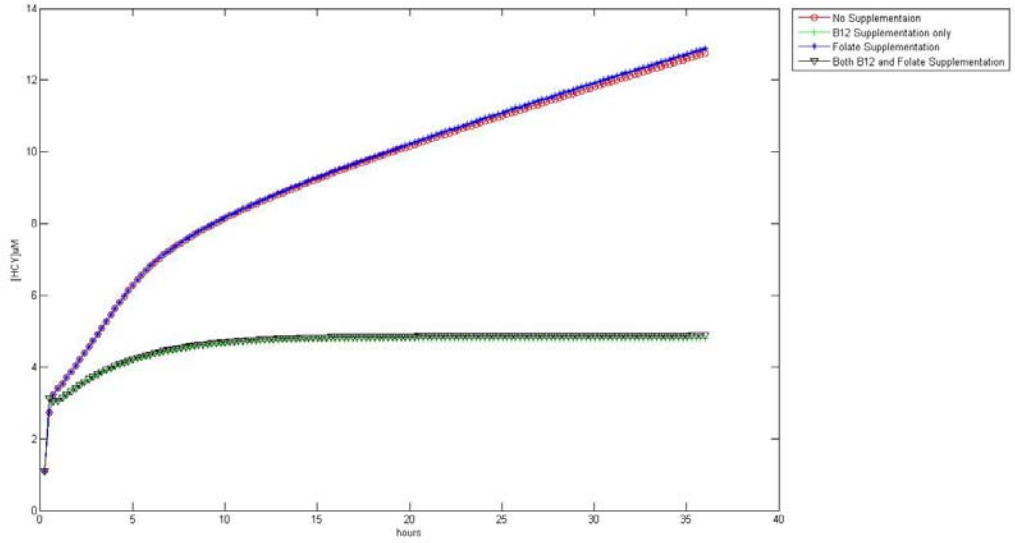

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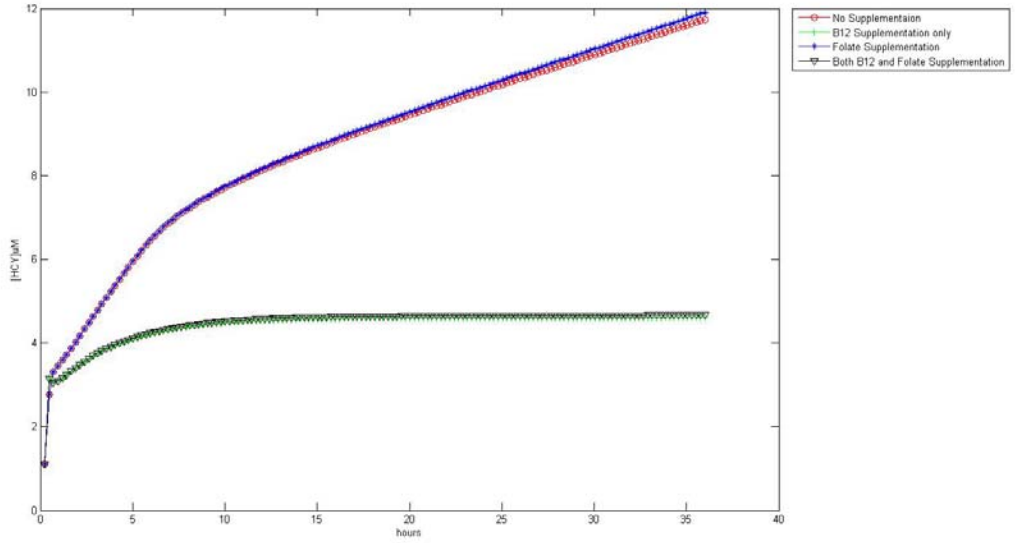

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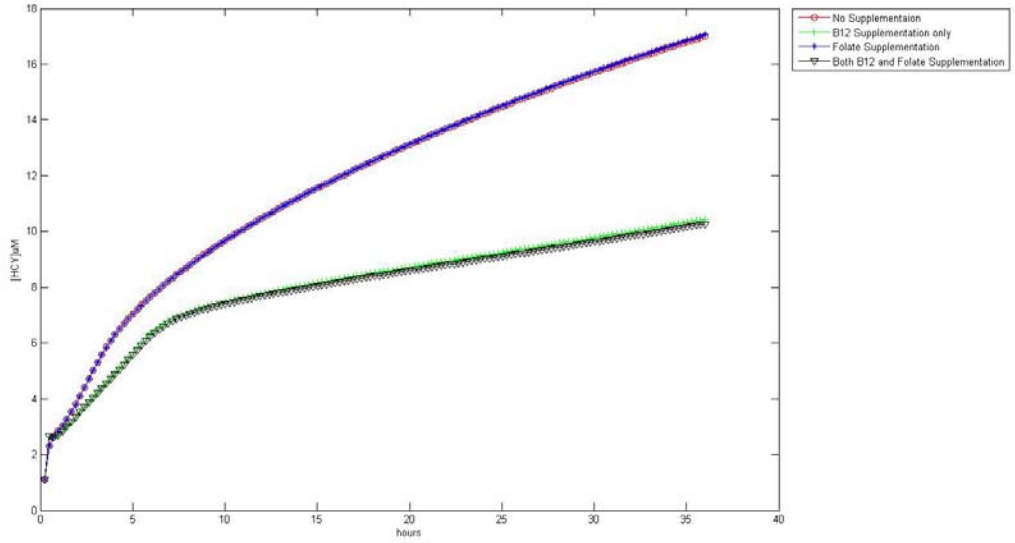

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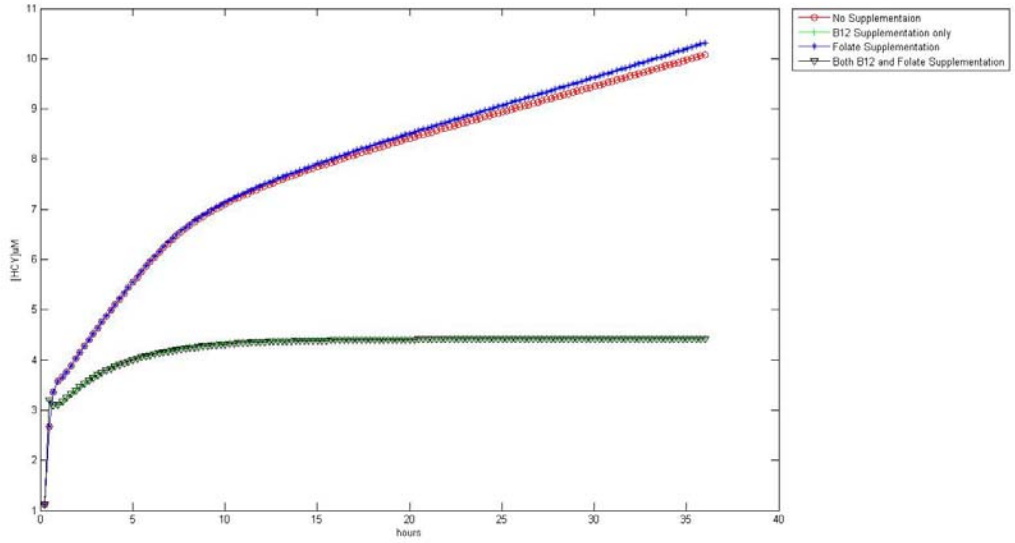

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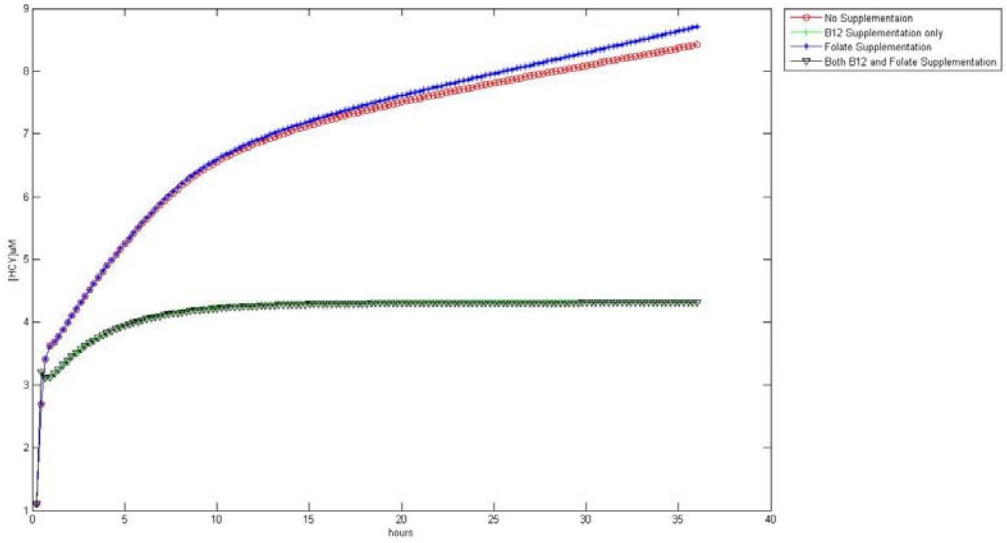

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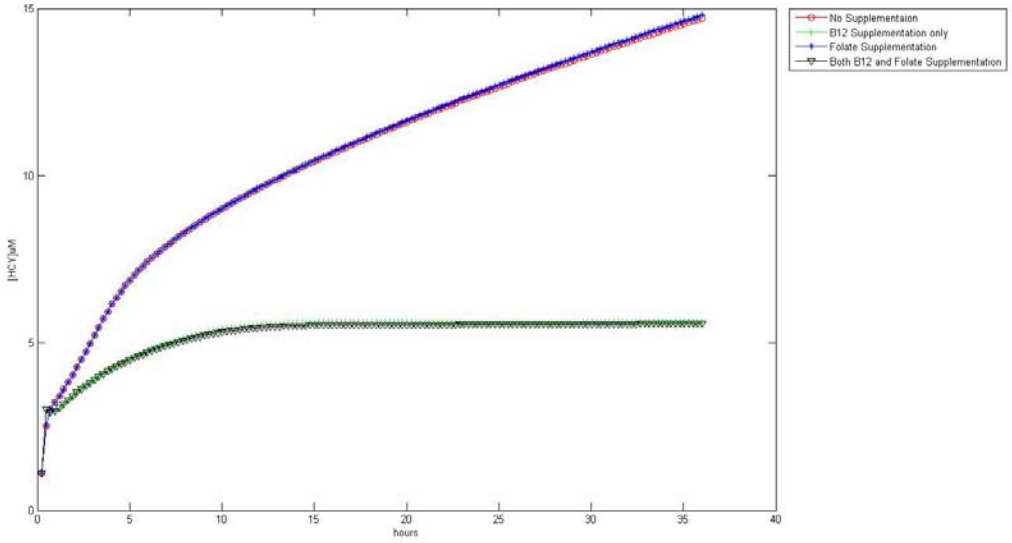

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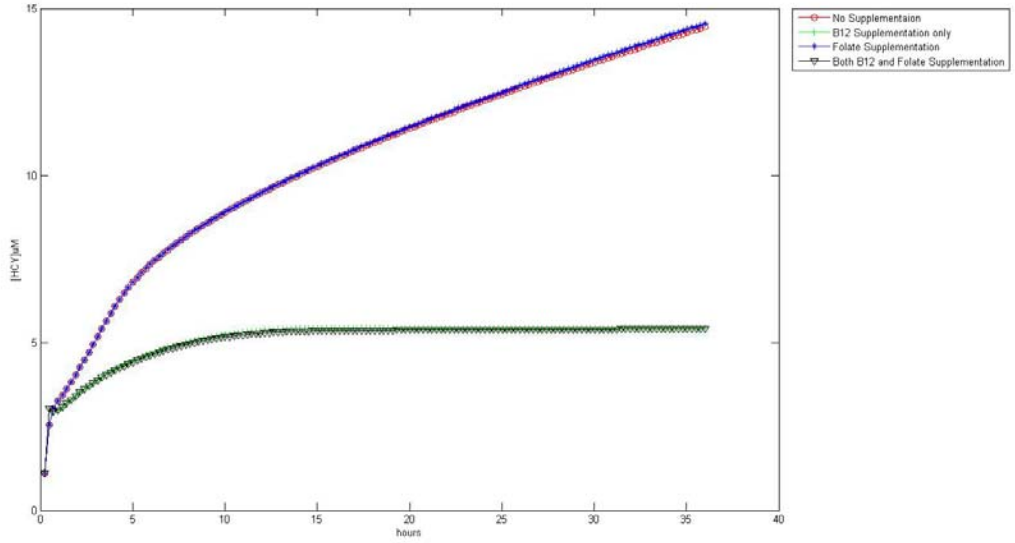

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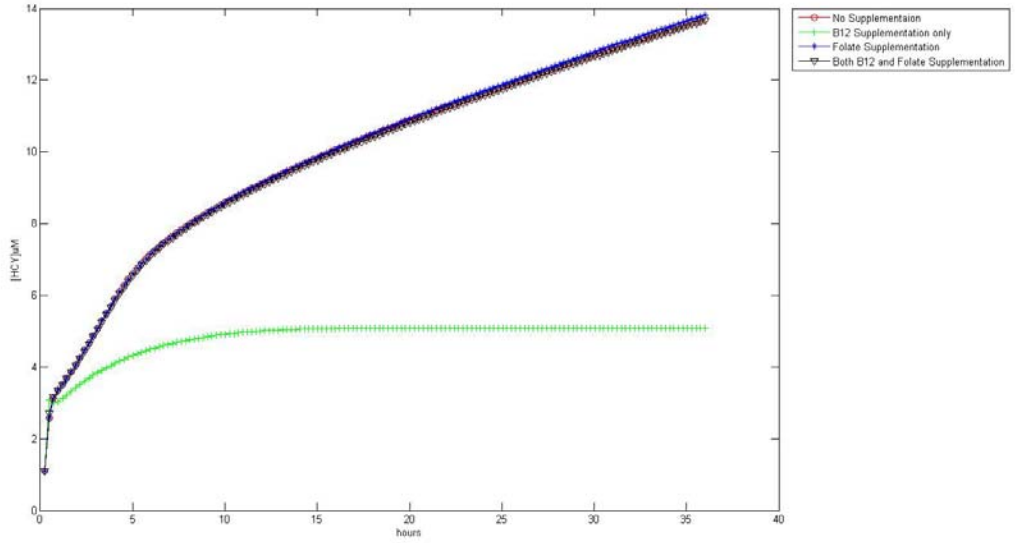

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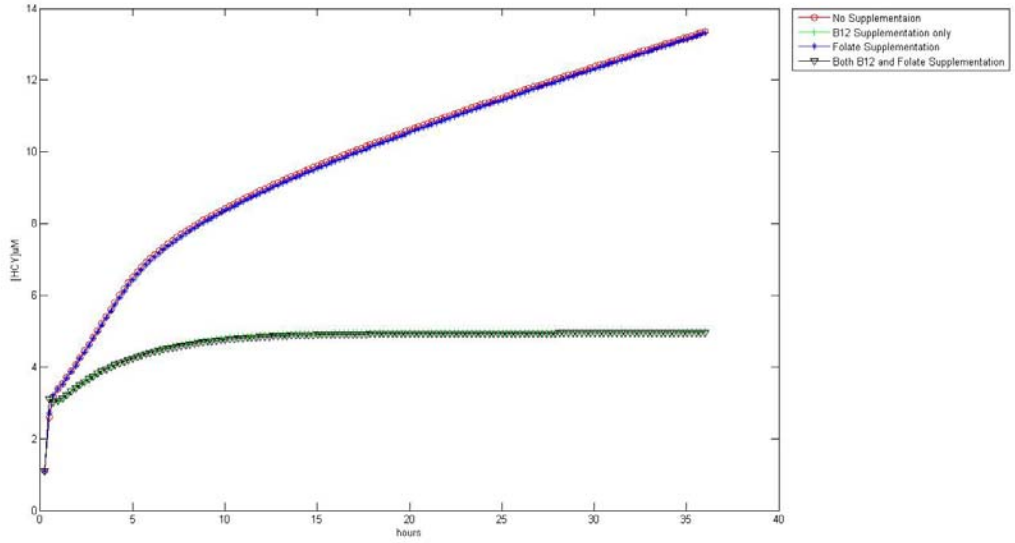

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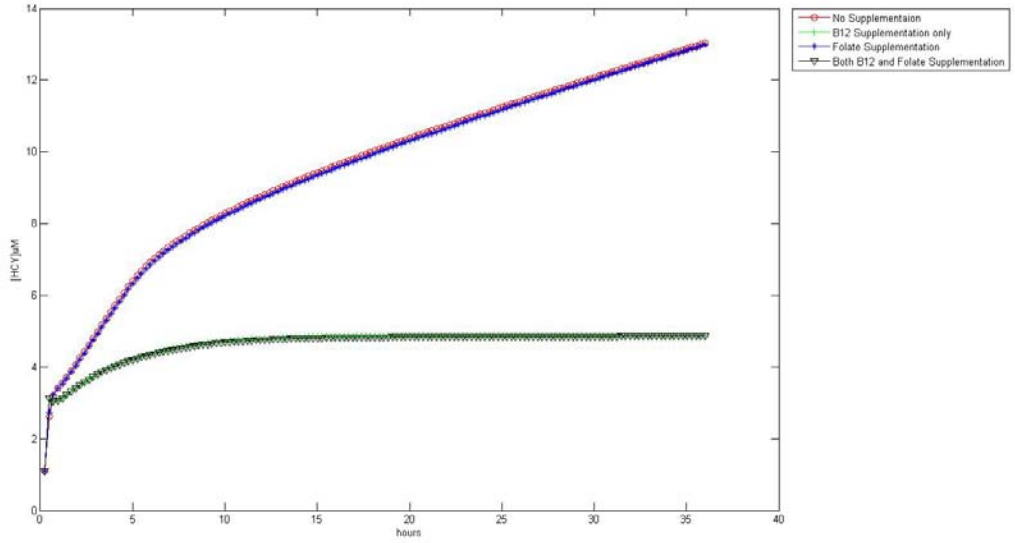

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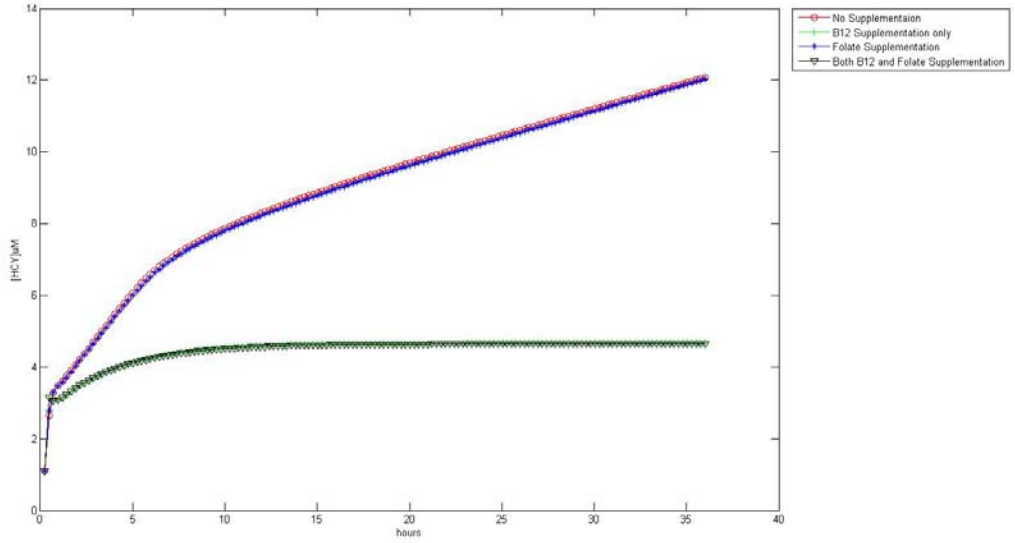

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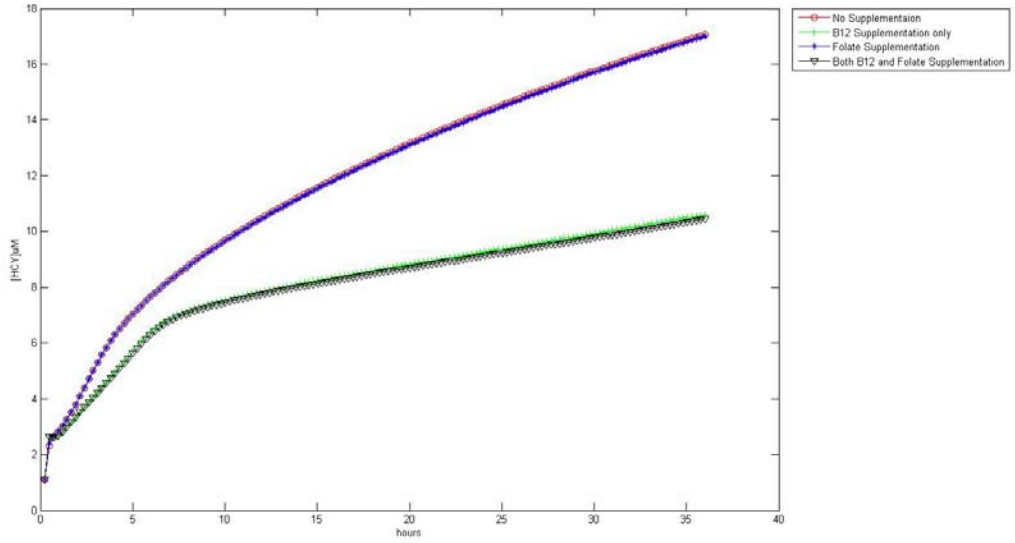

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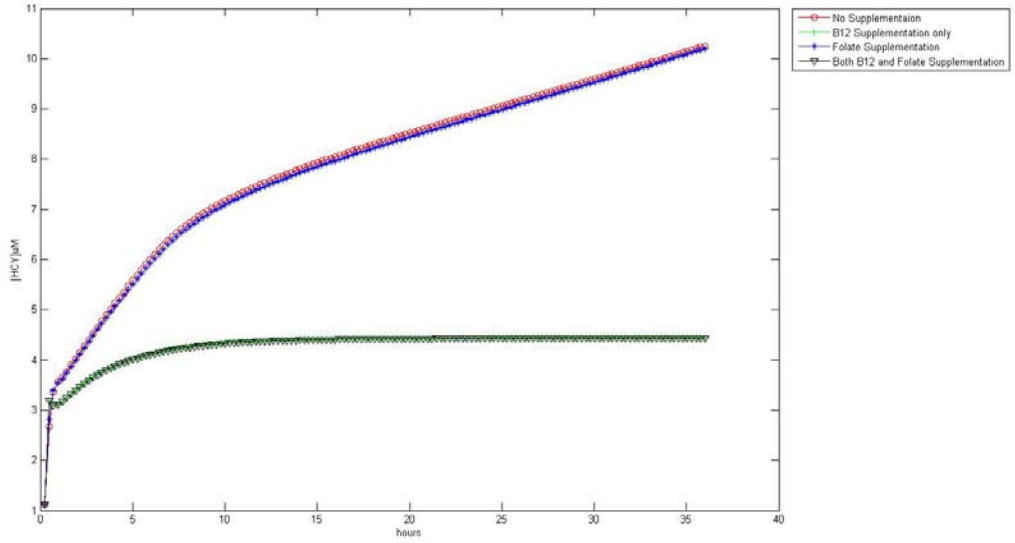

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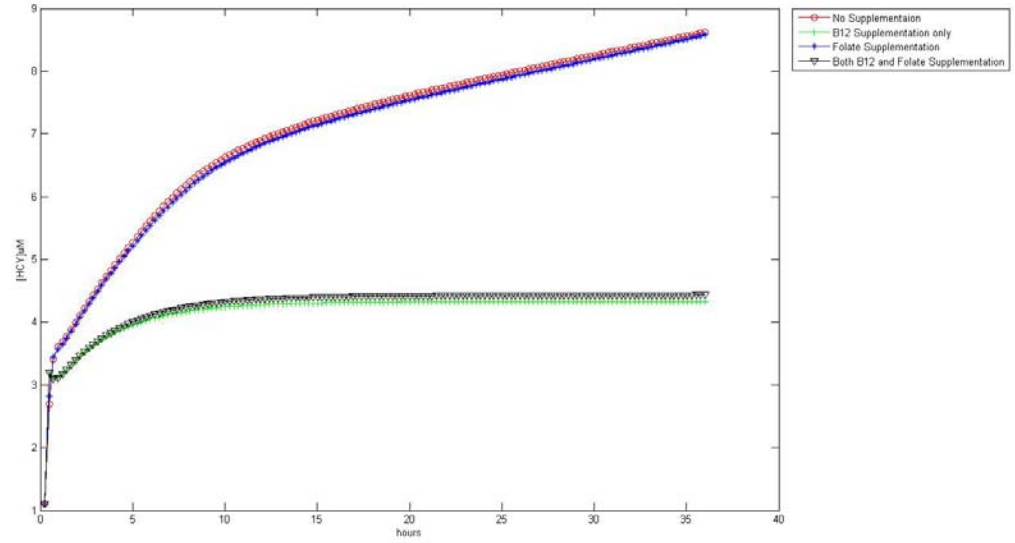

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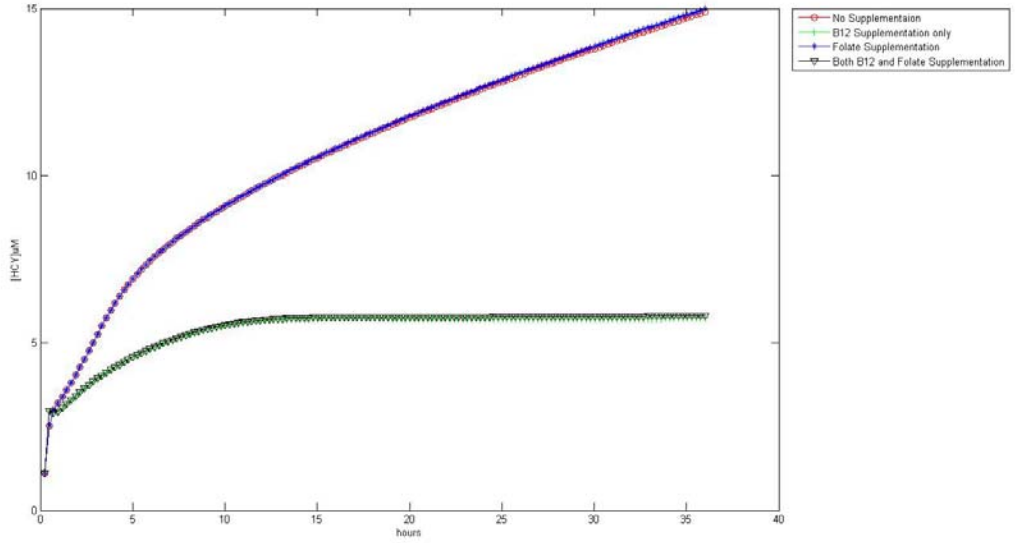

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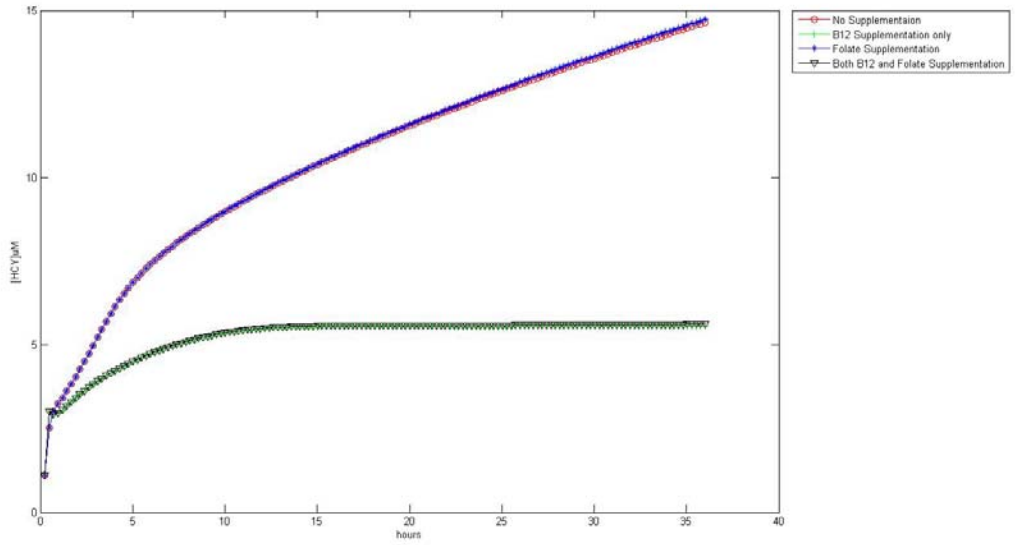

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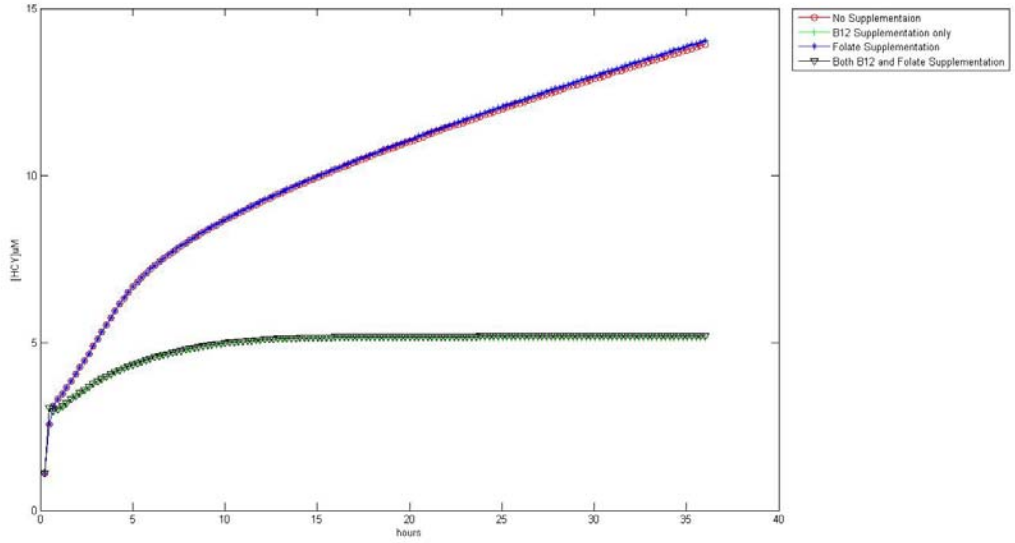

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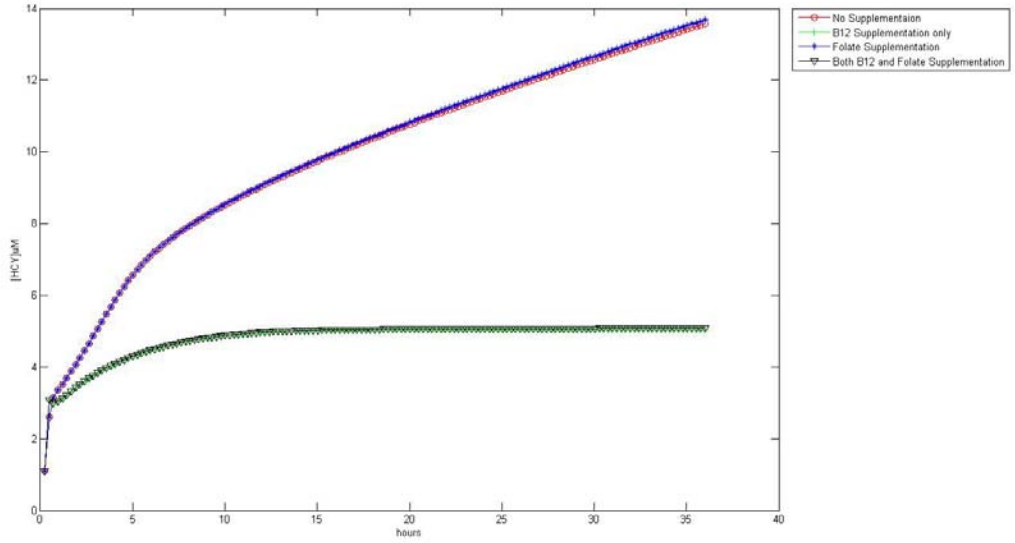

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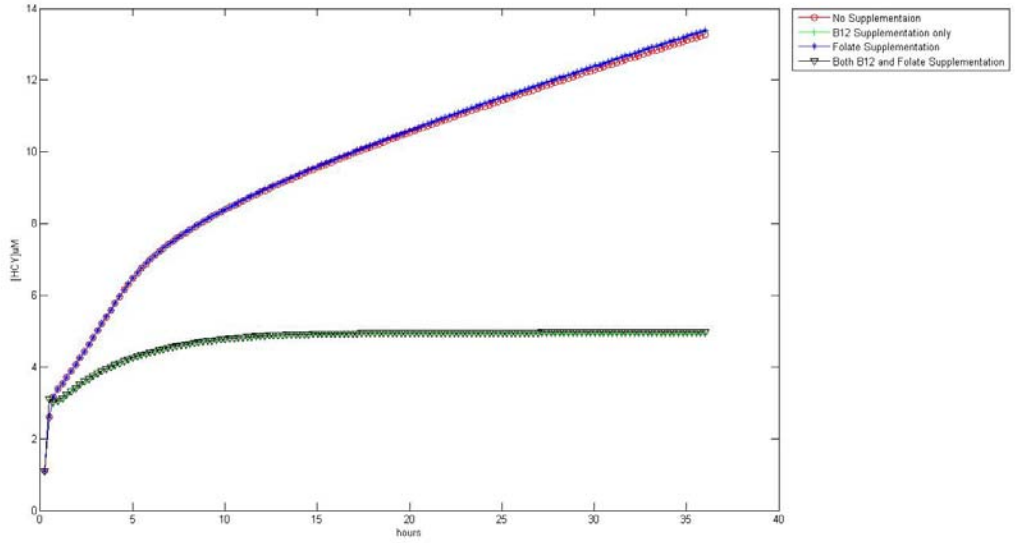

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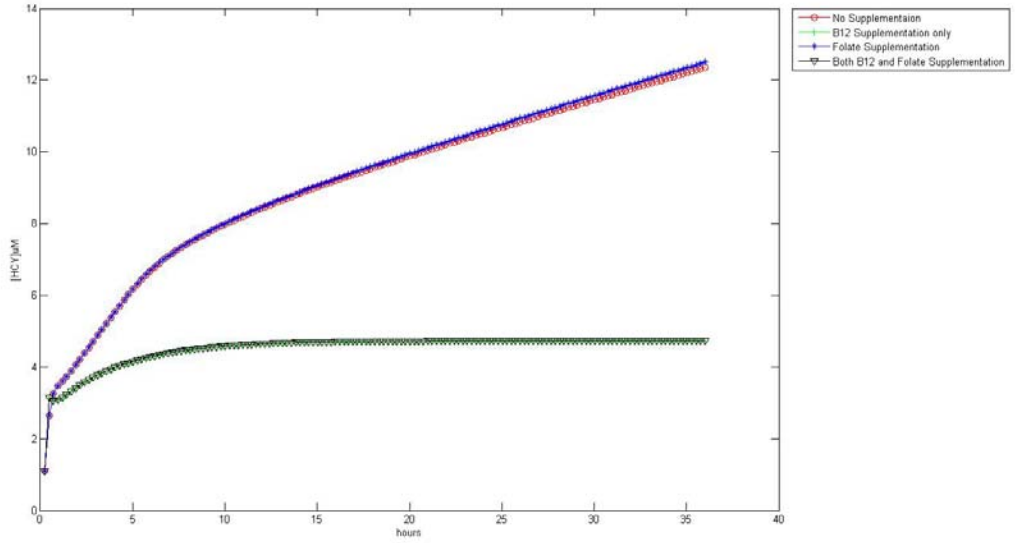

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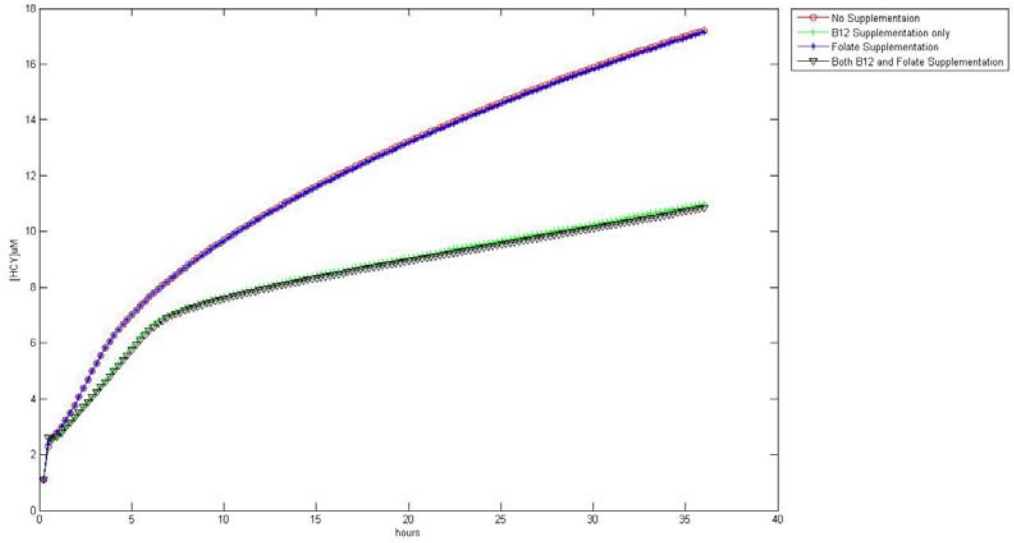

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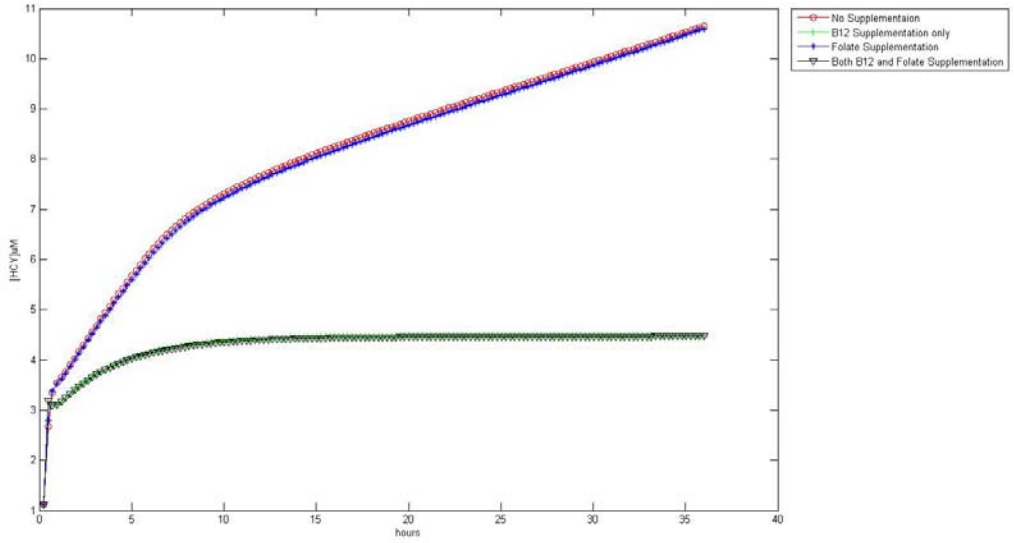

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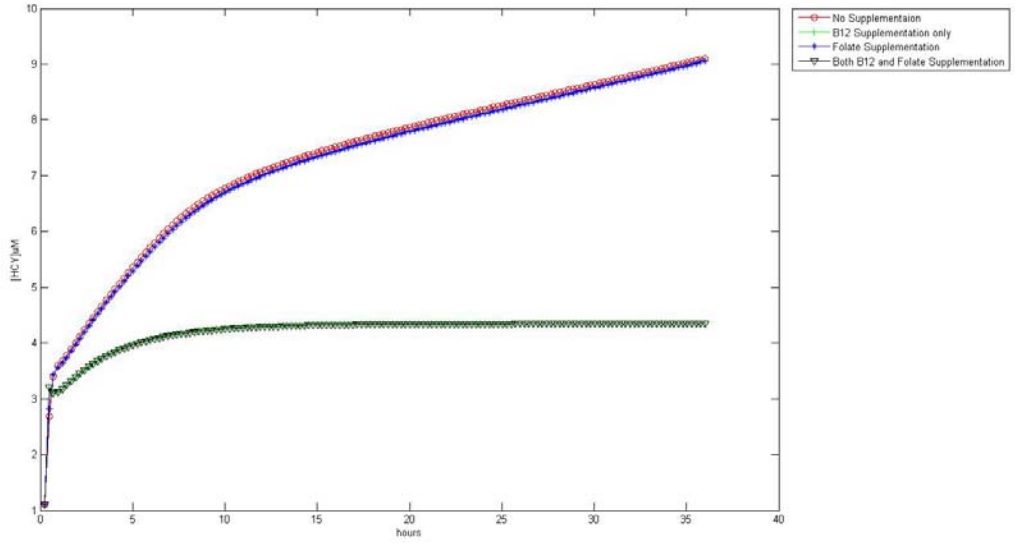

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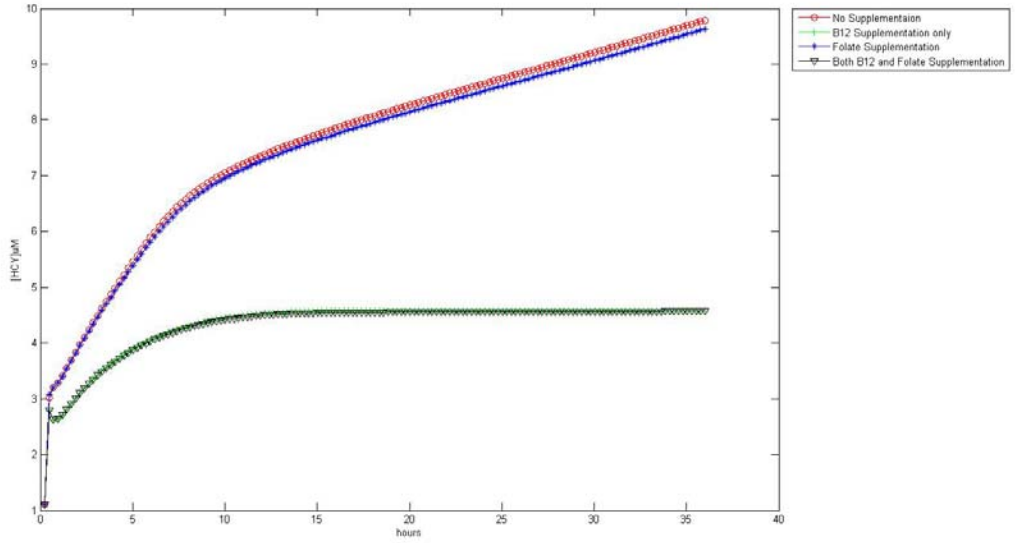

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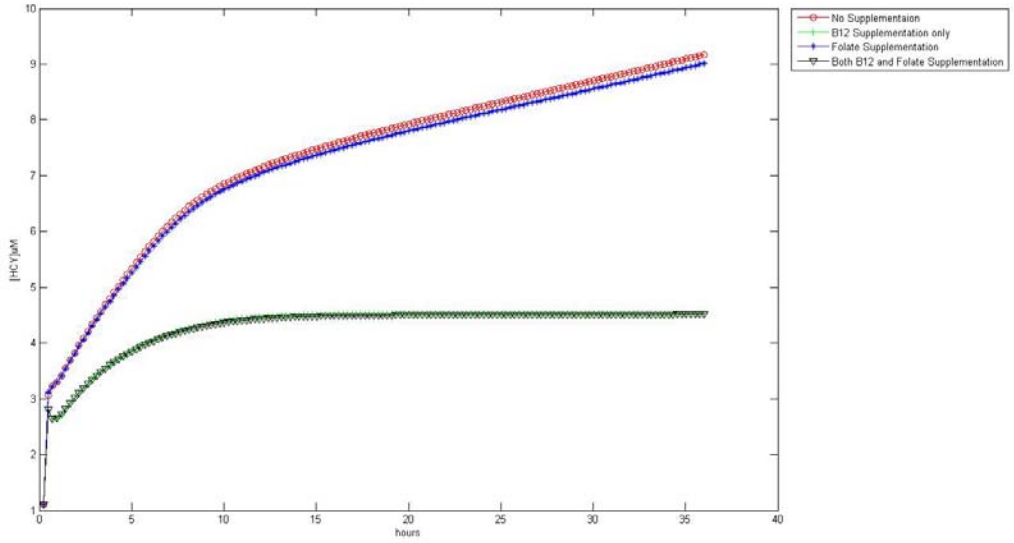

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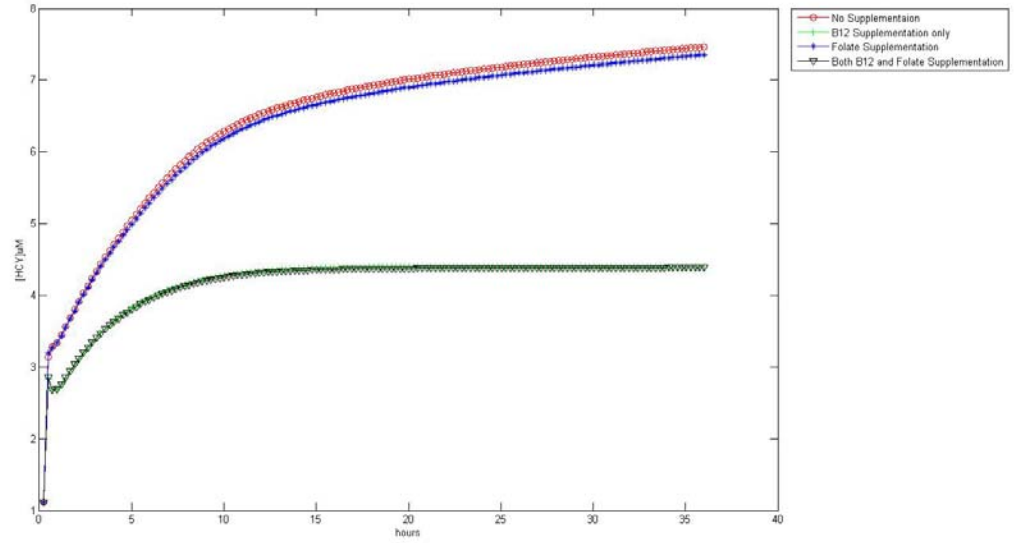

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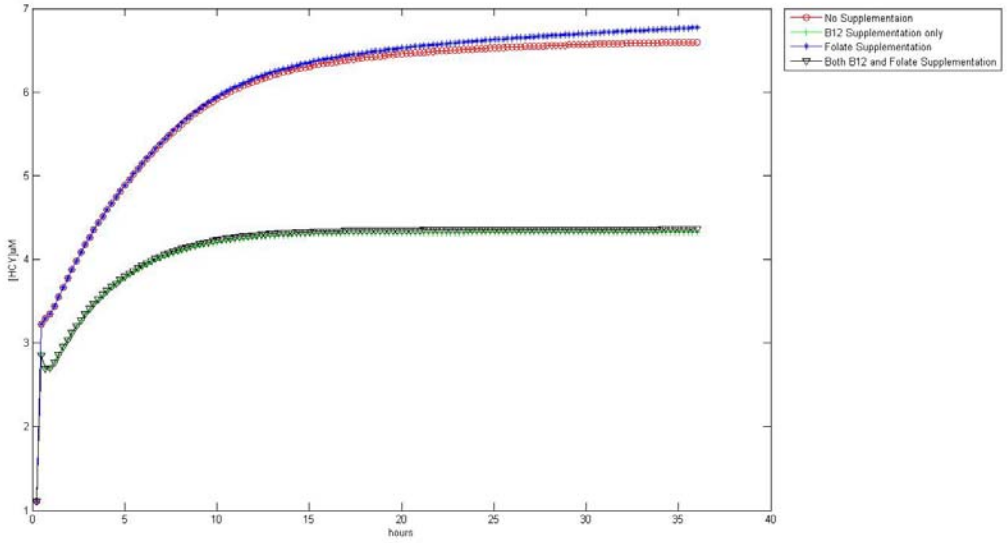

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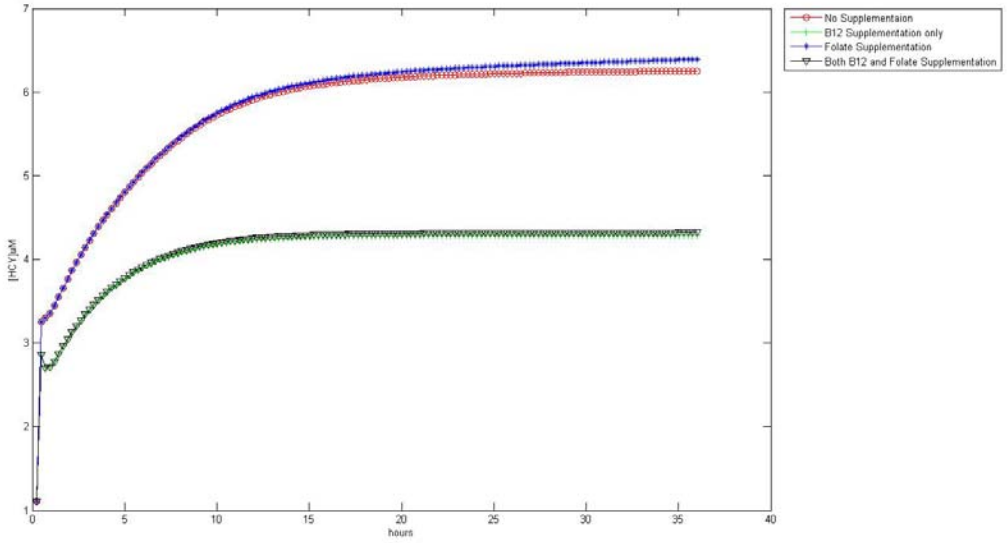

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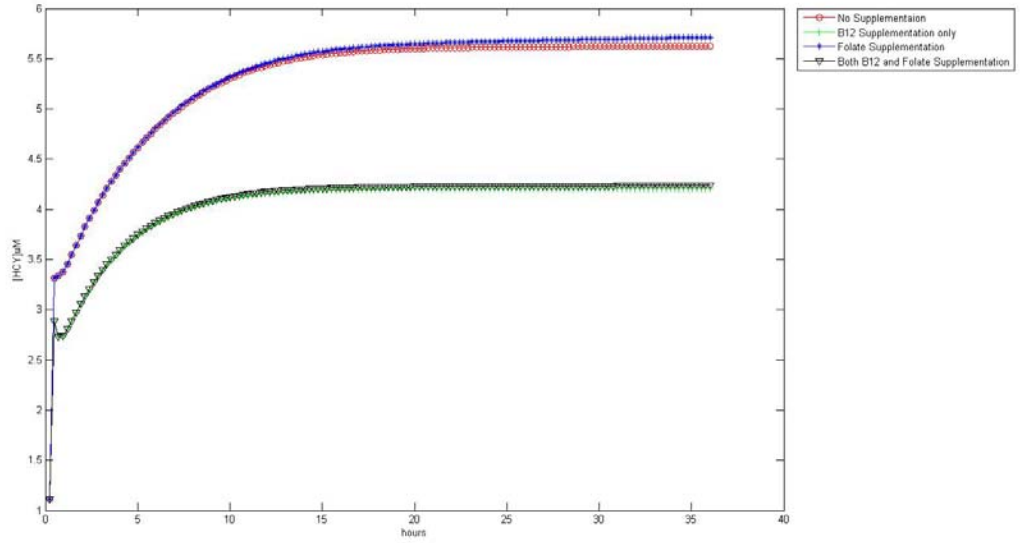
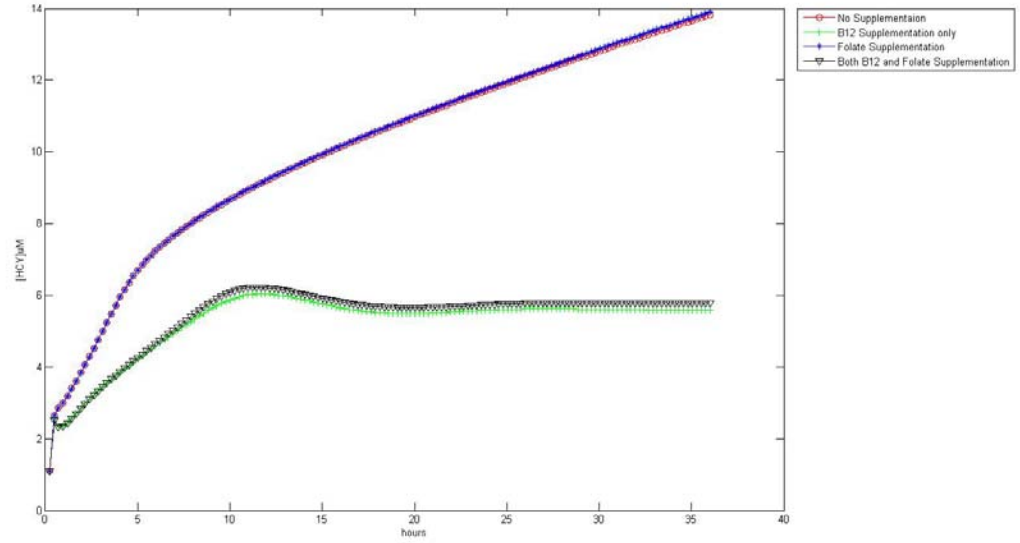


MTHFR 677 CT, MTHFR 1298 CC, MTHFD1 1958 GG, MTR 2756 AA, cSHMT 1420 CT



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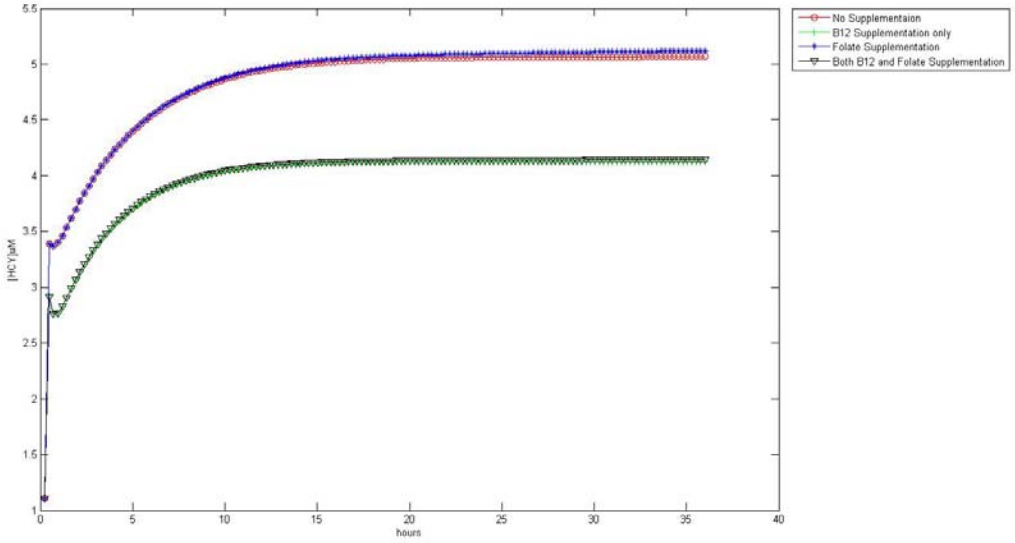

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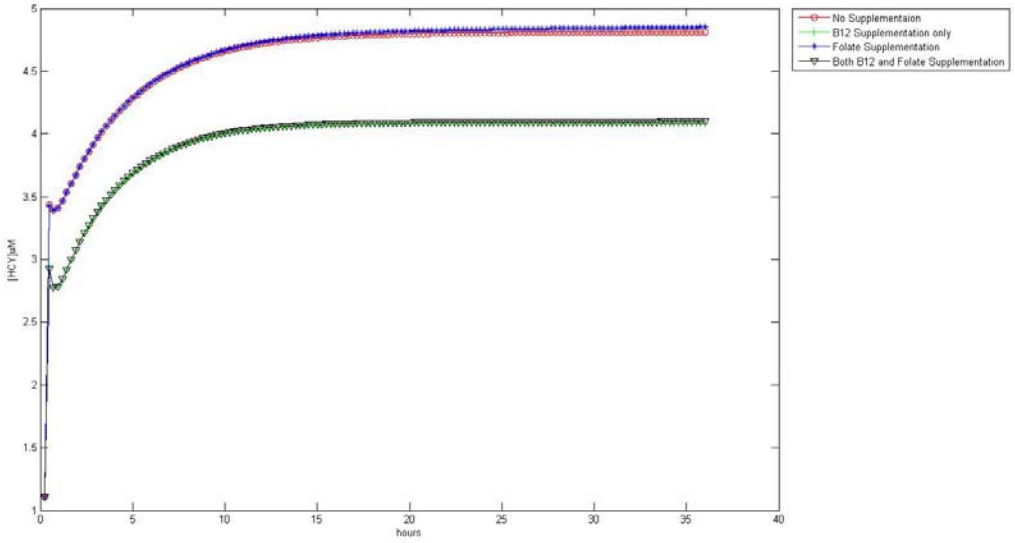

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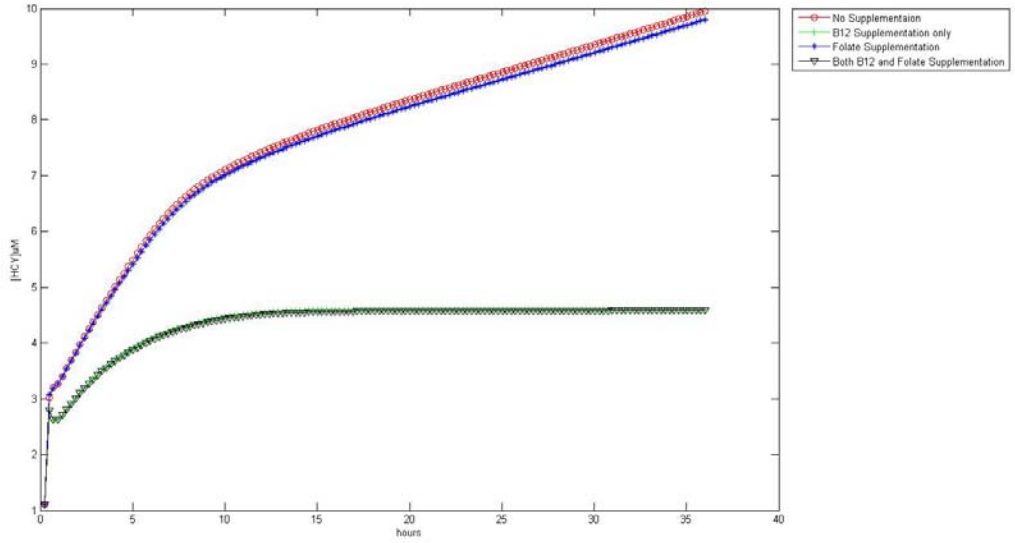

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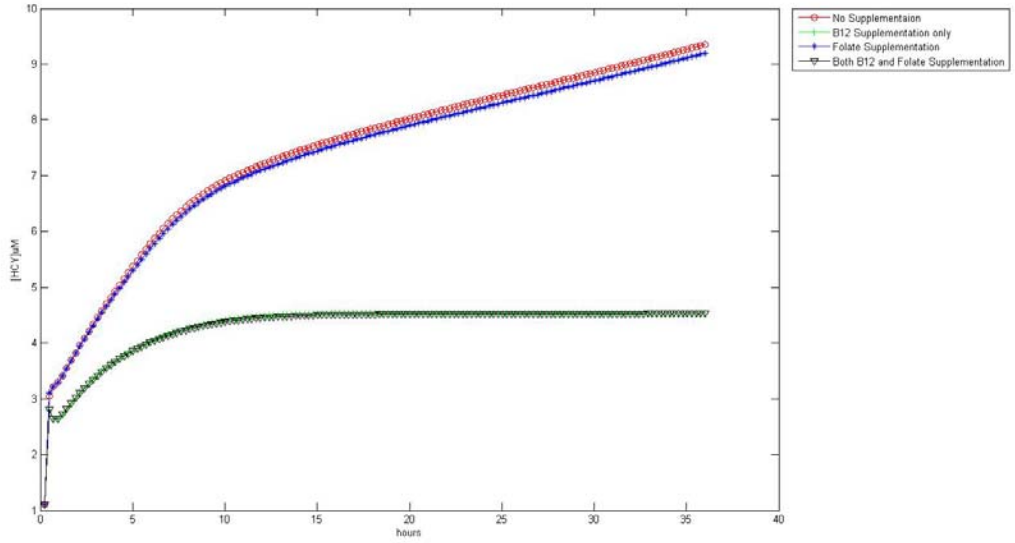

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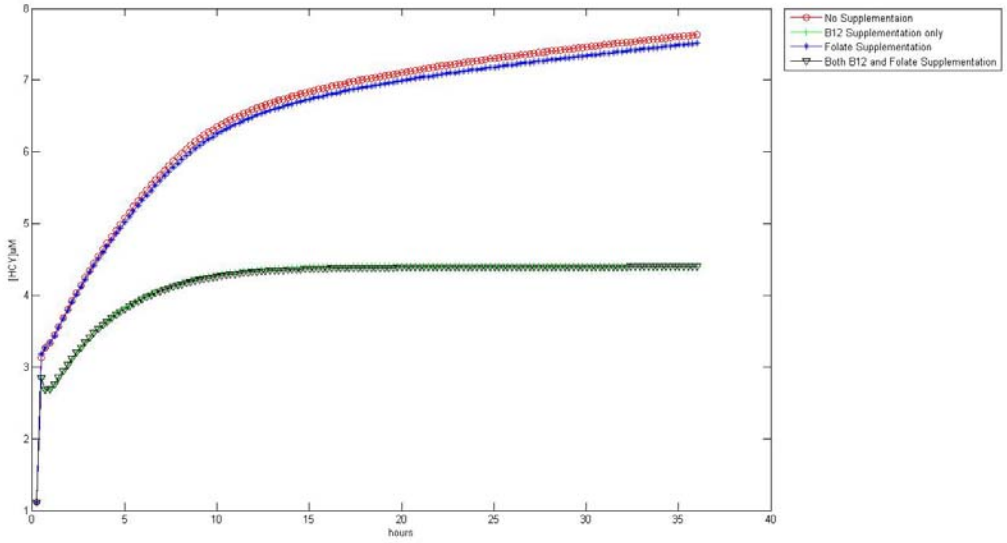

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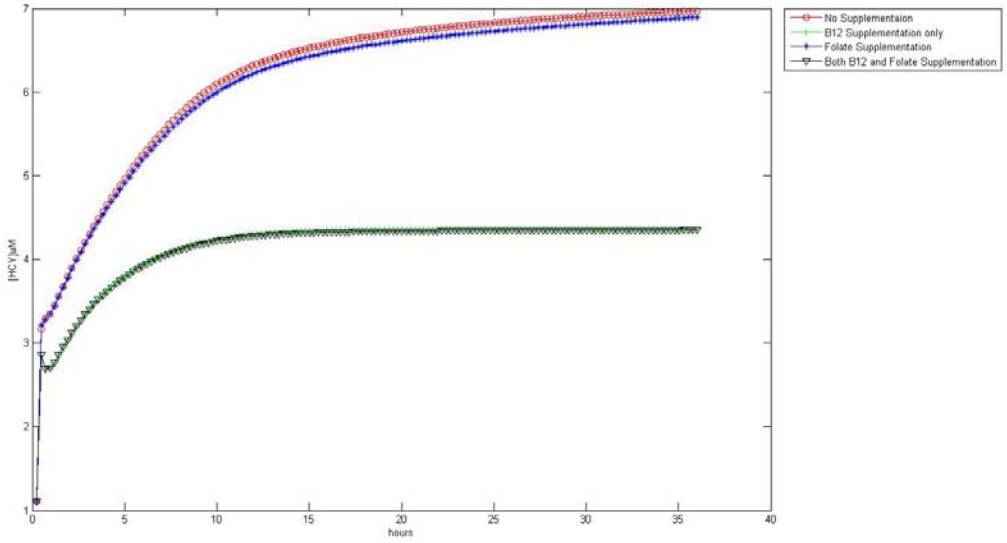

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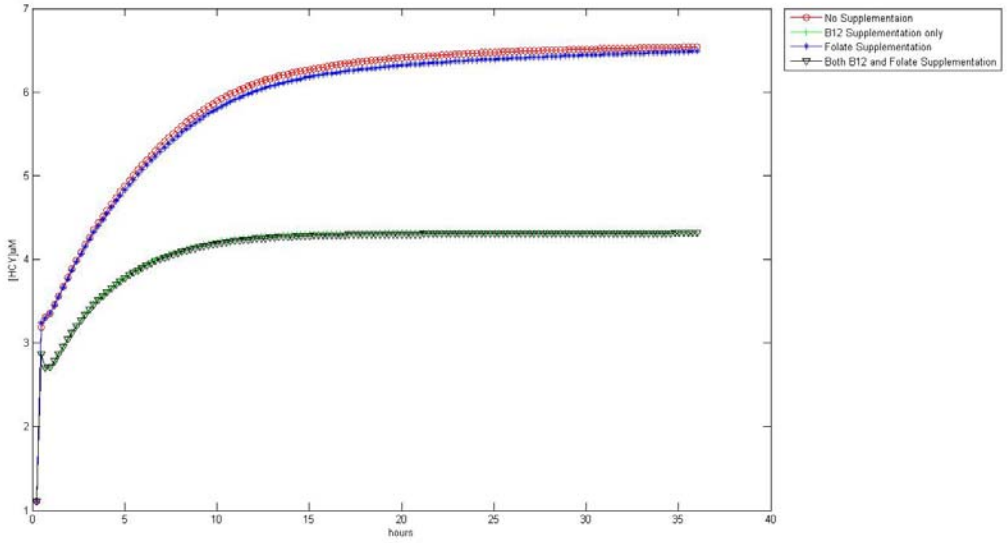

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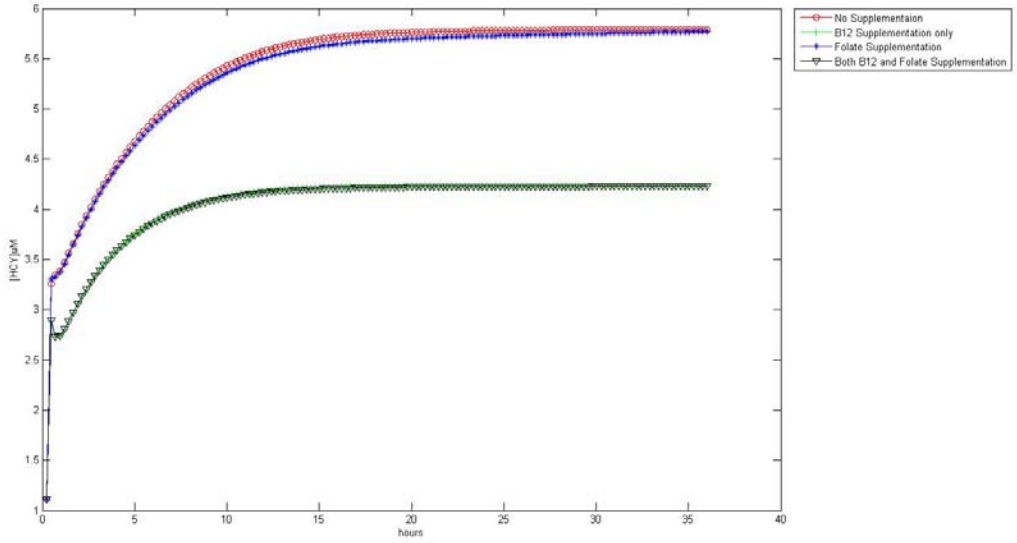

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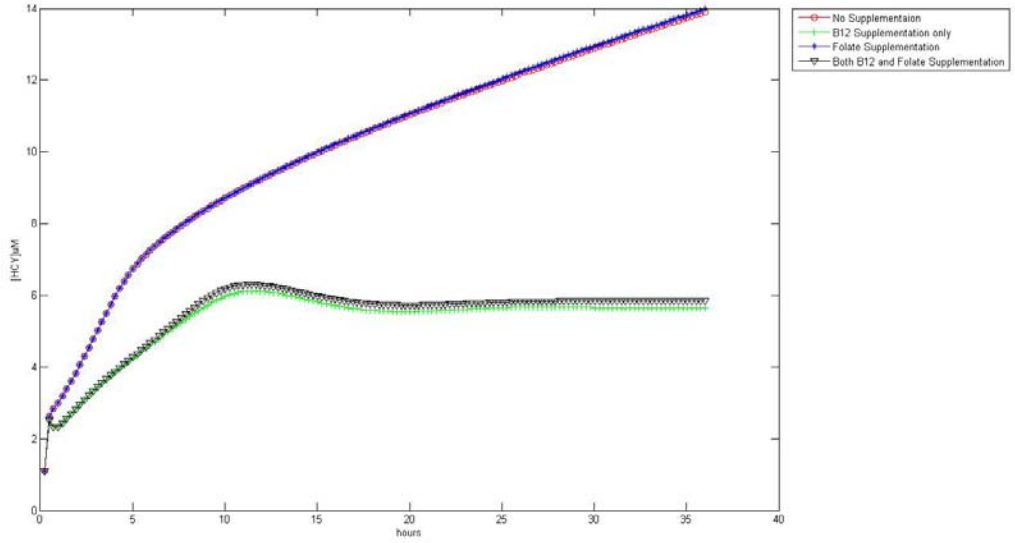

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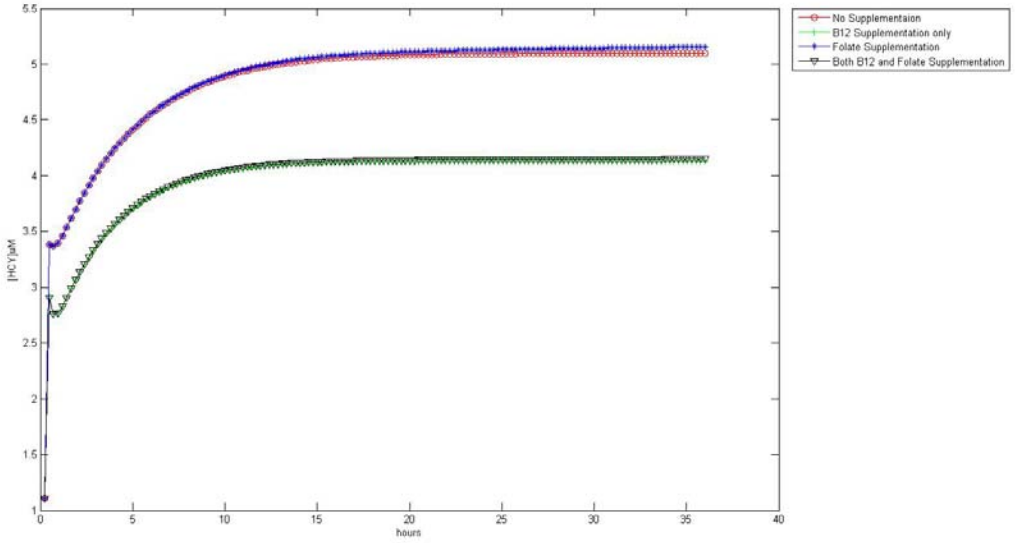

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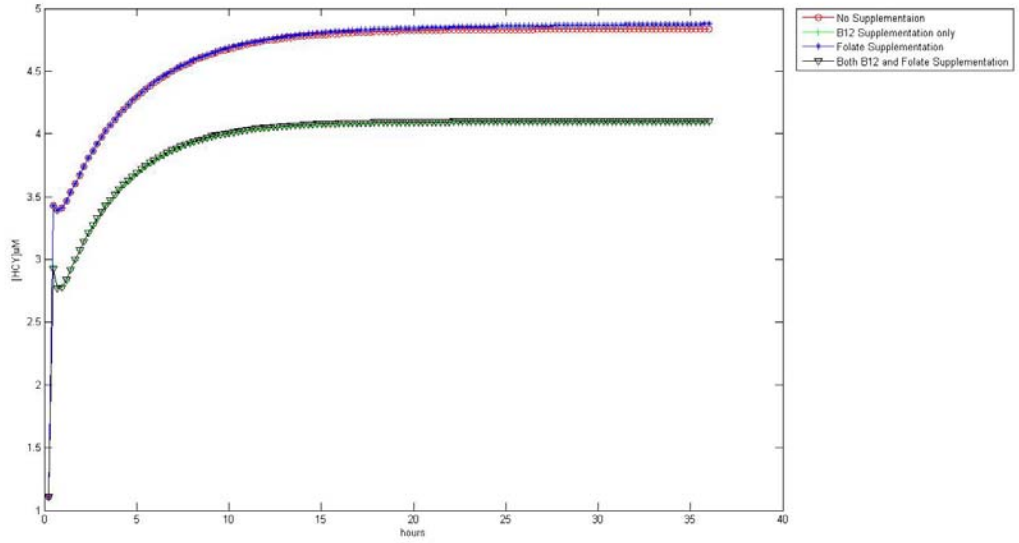

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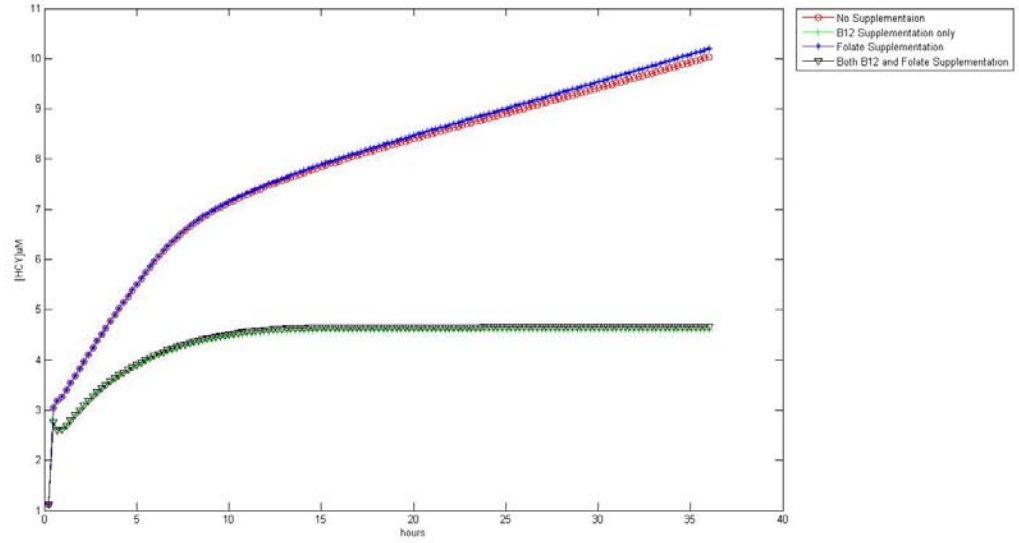

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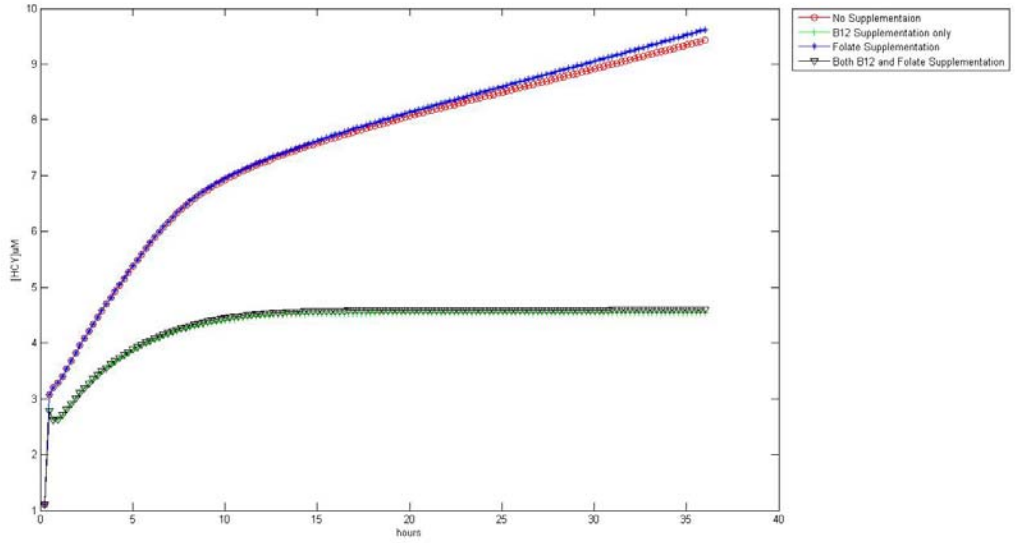

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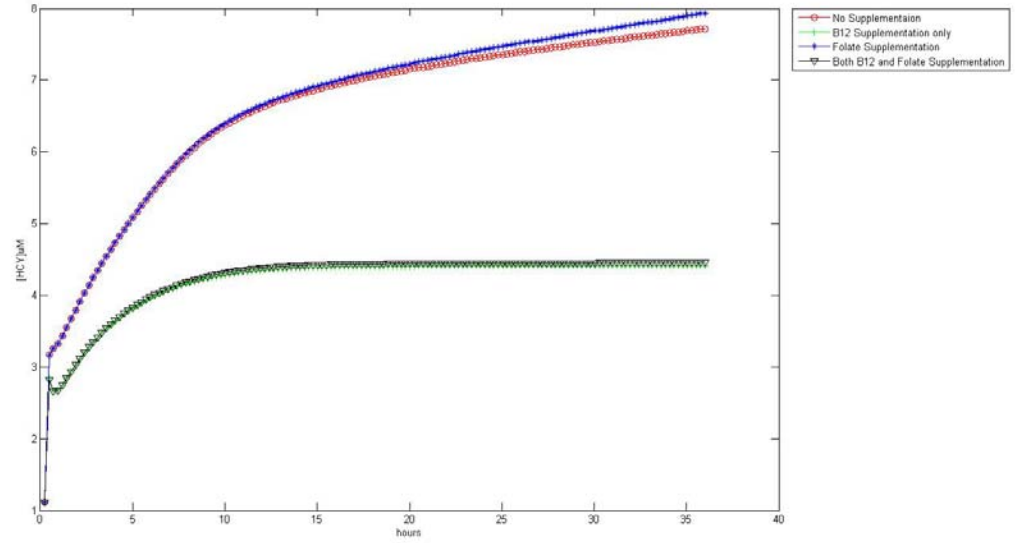

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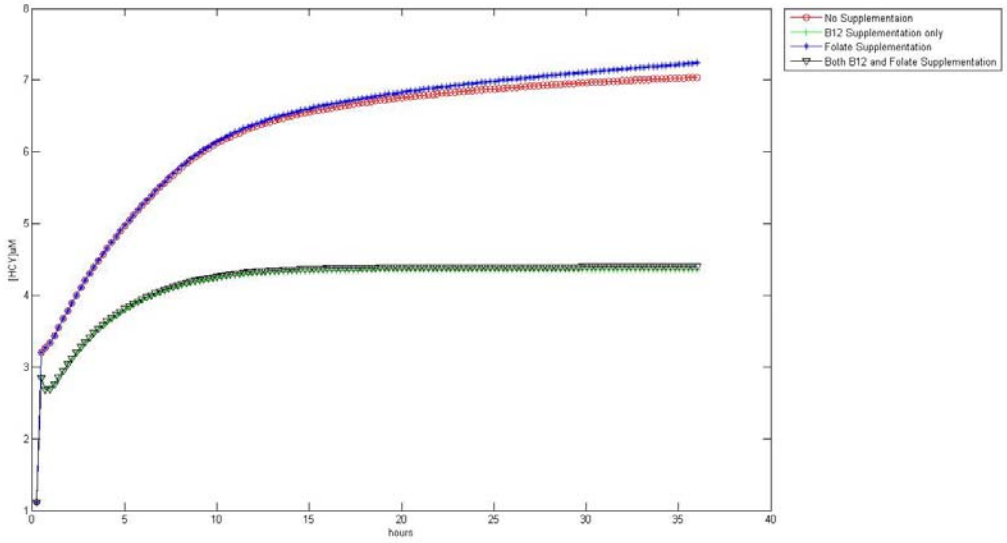

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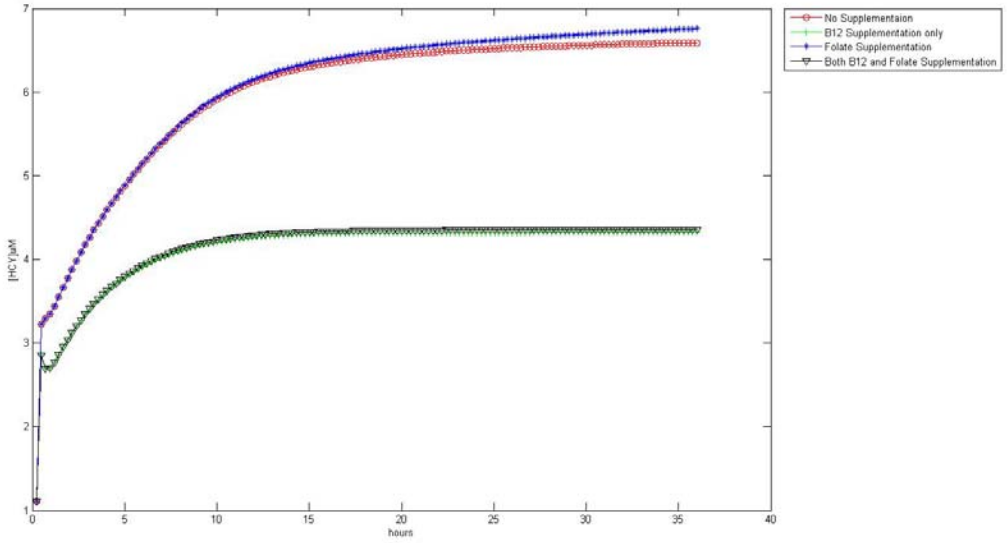

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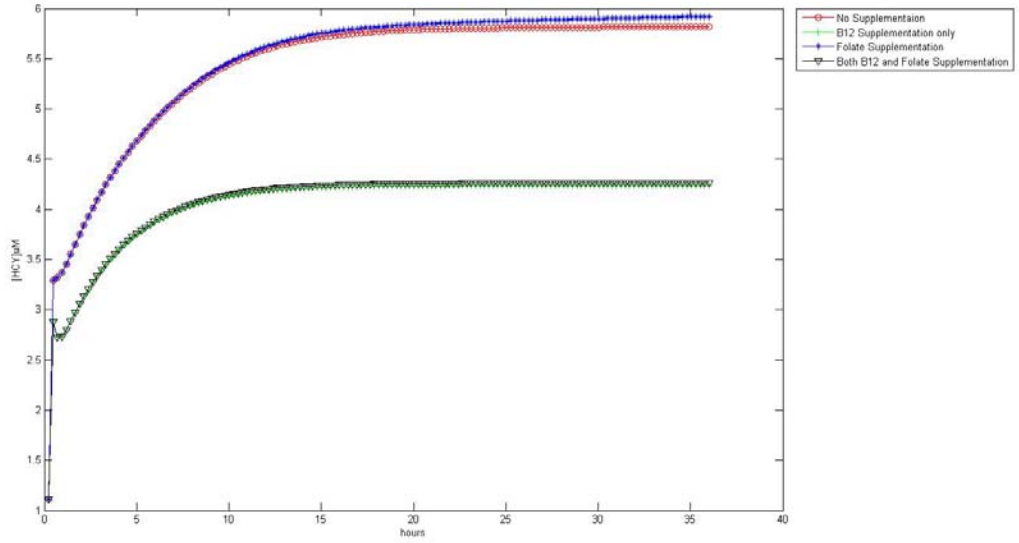

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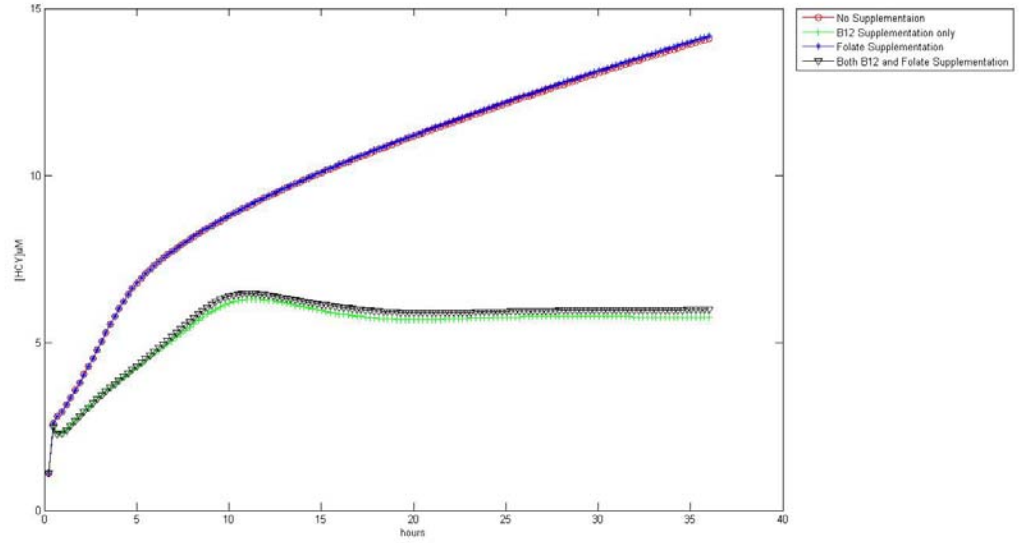

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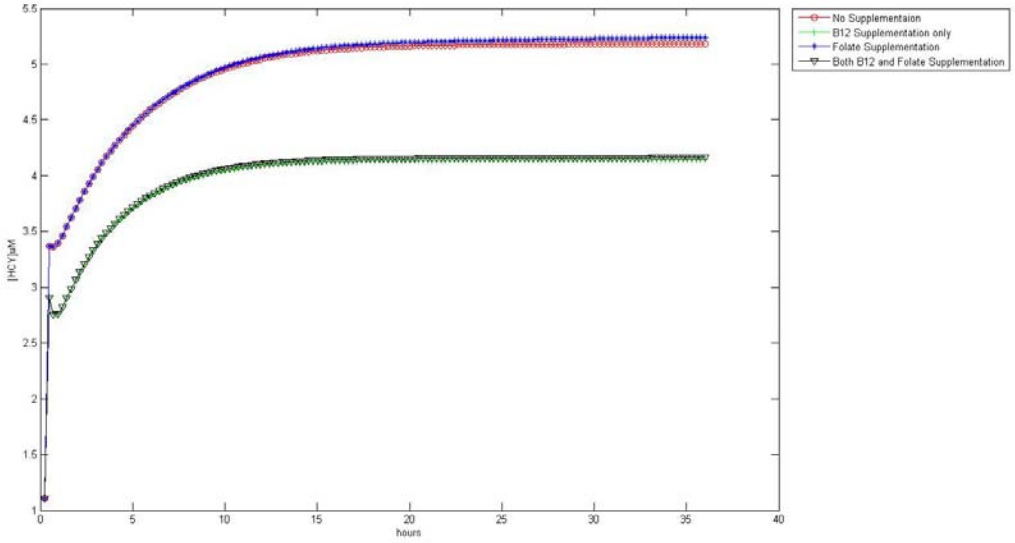

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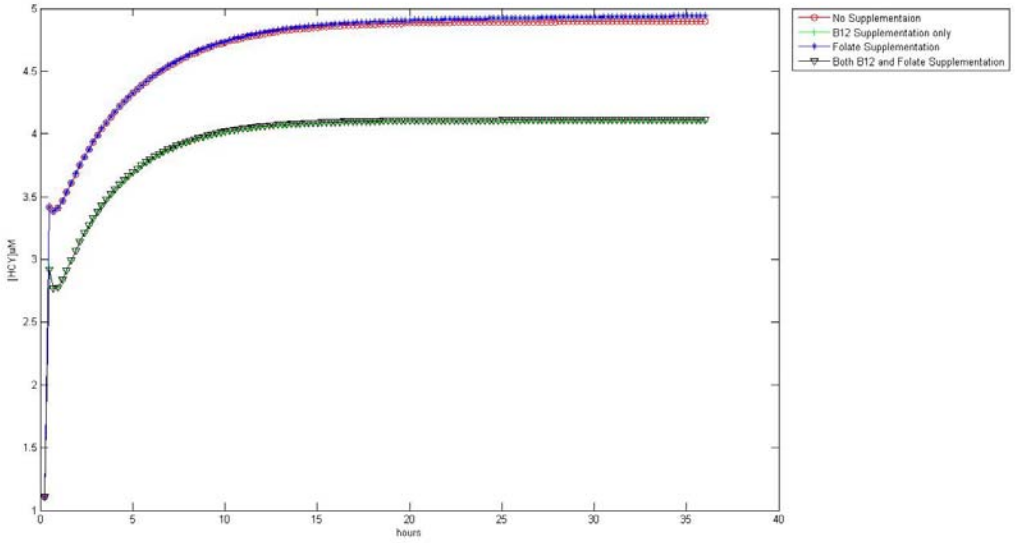

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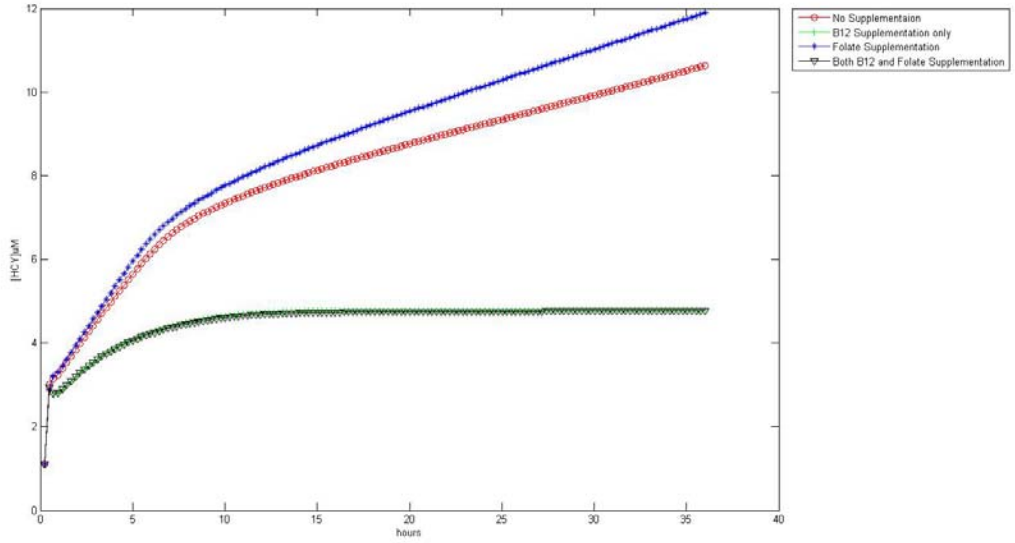

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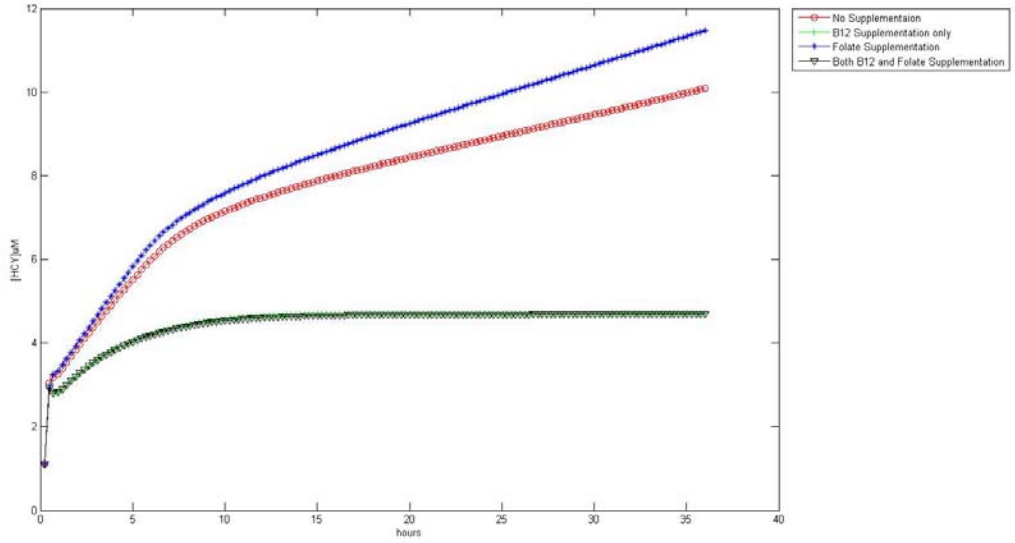

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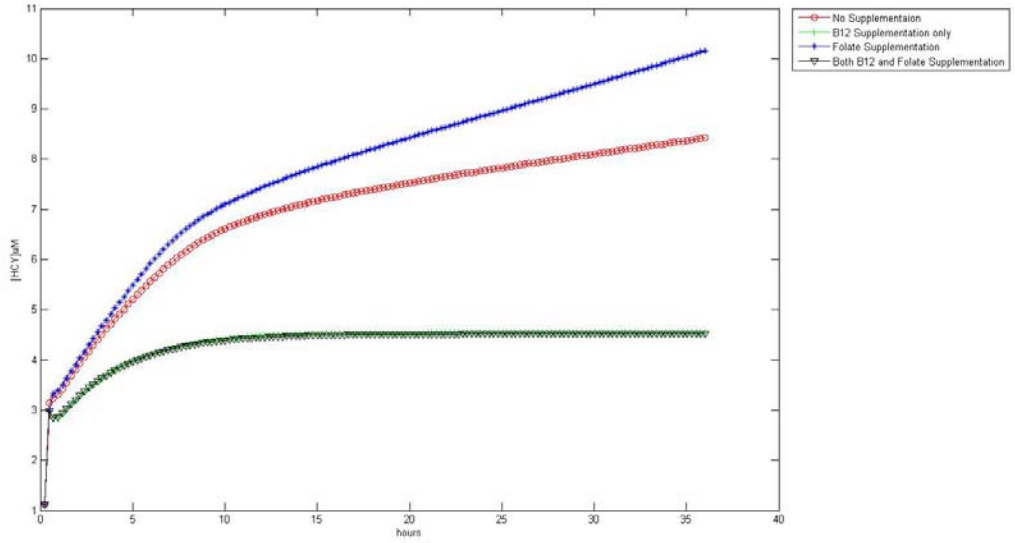

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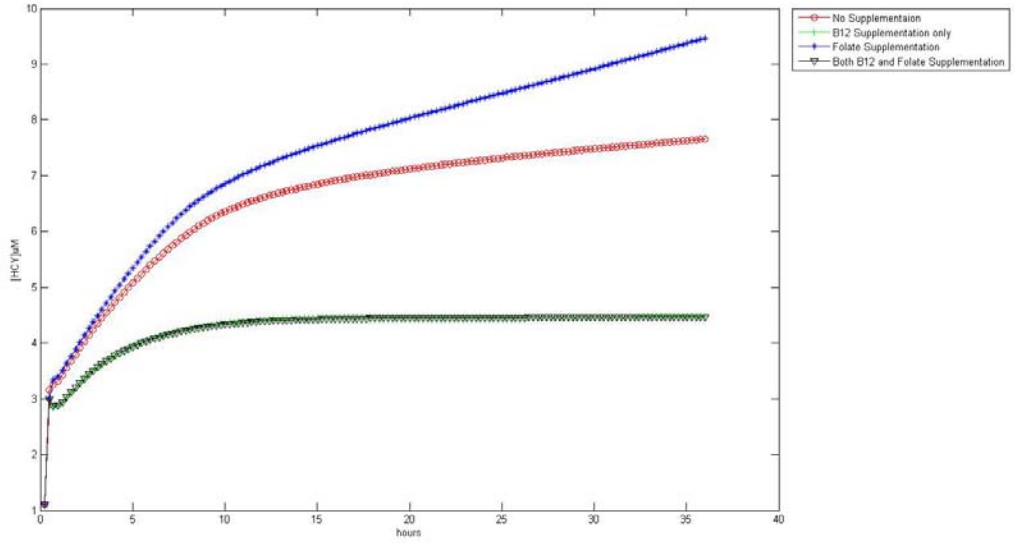

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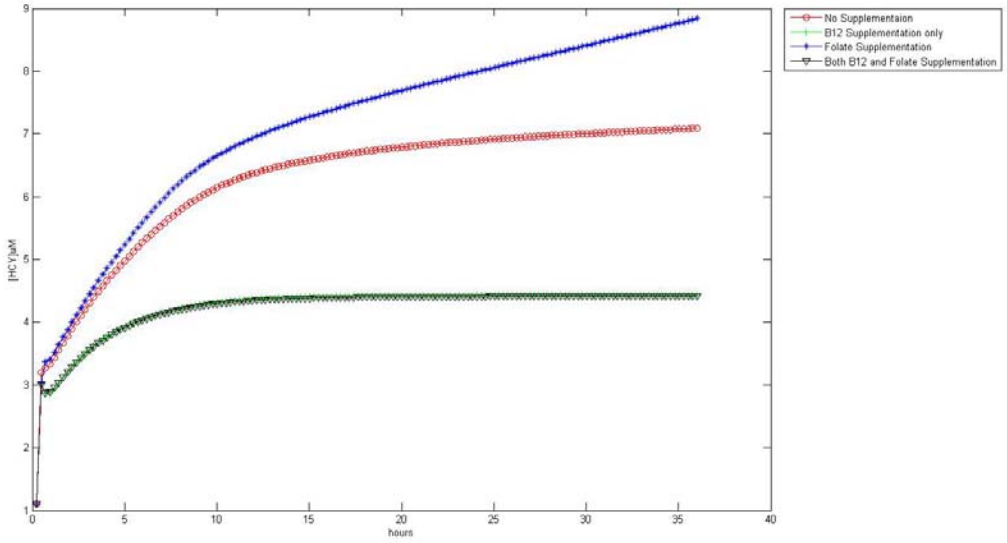

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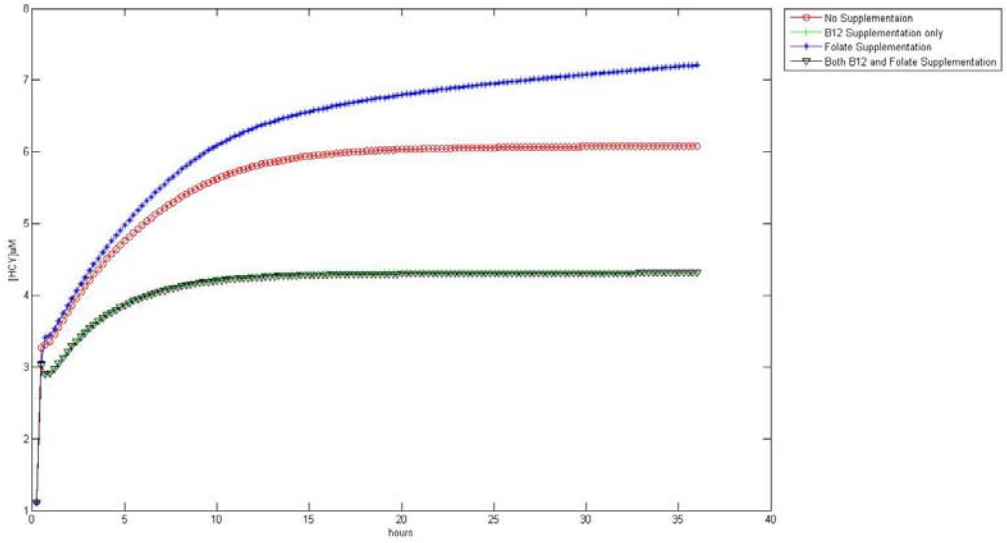

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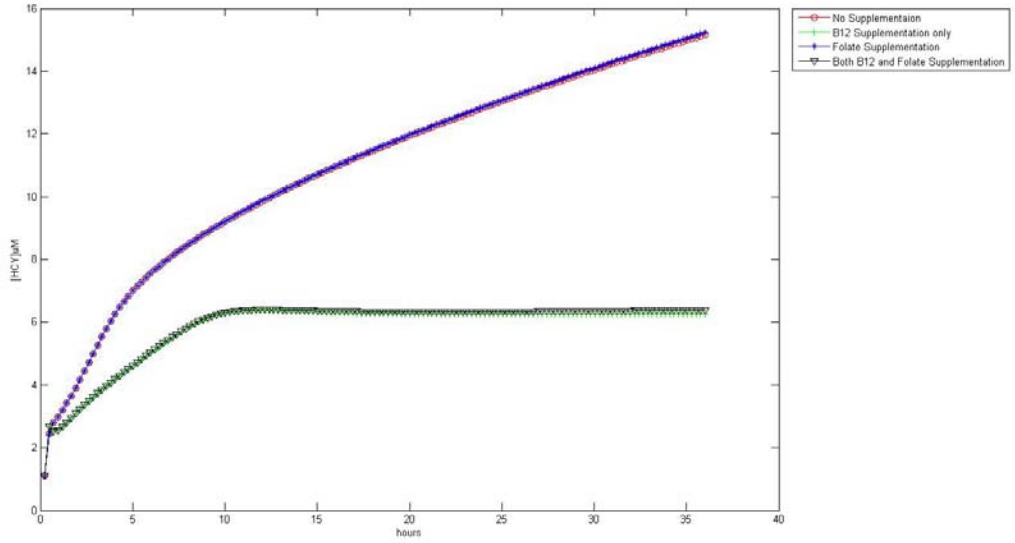

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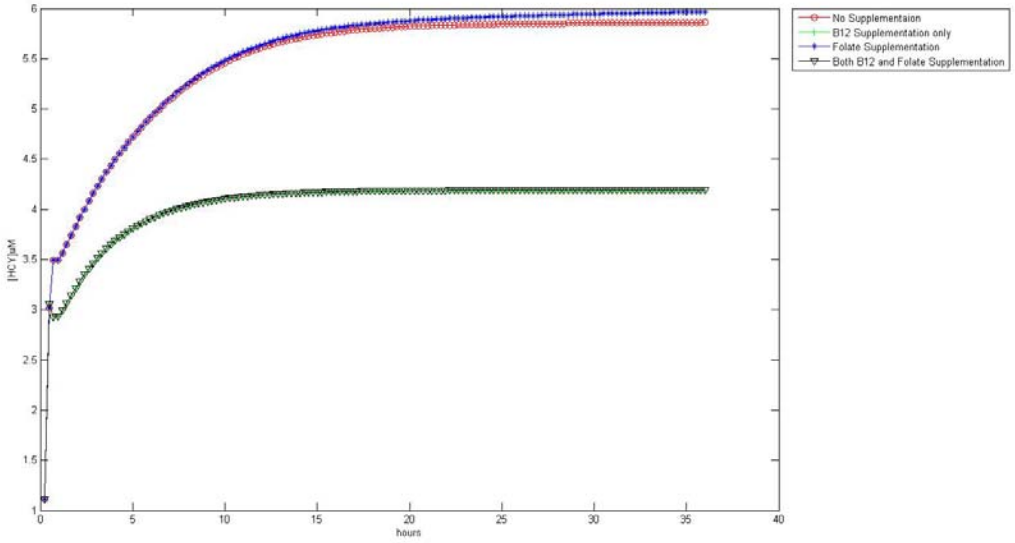

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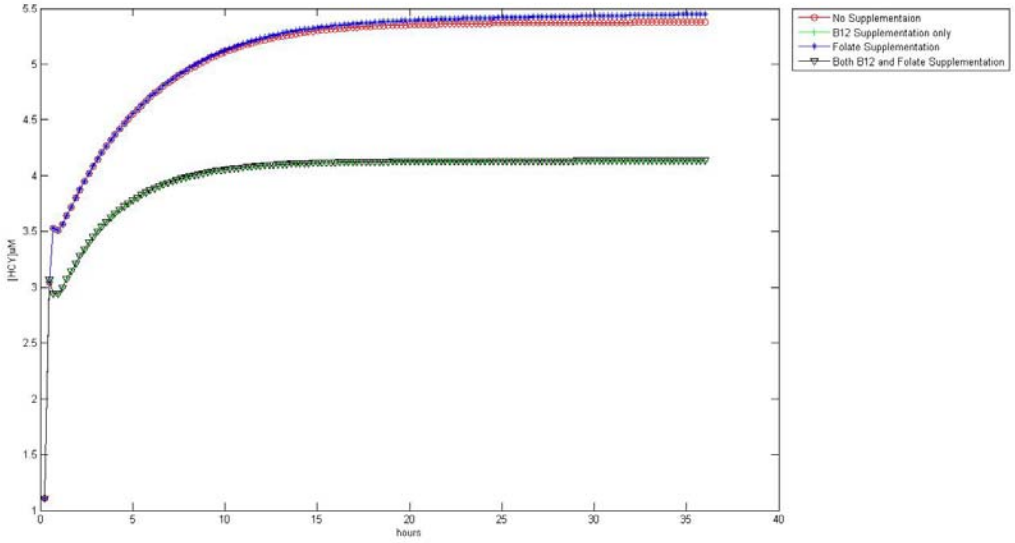

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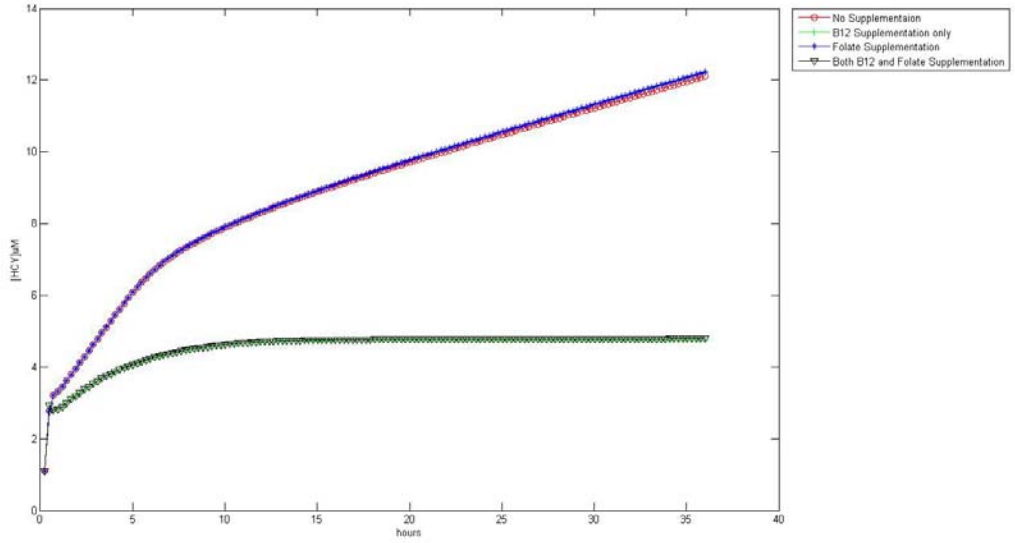

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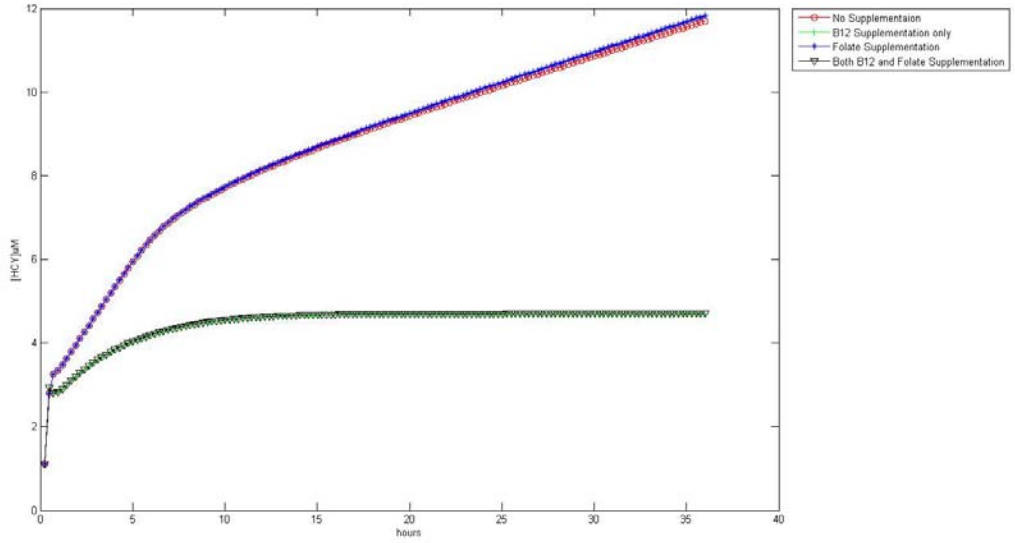

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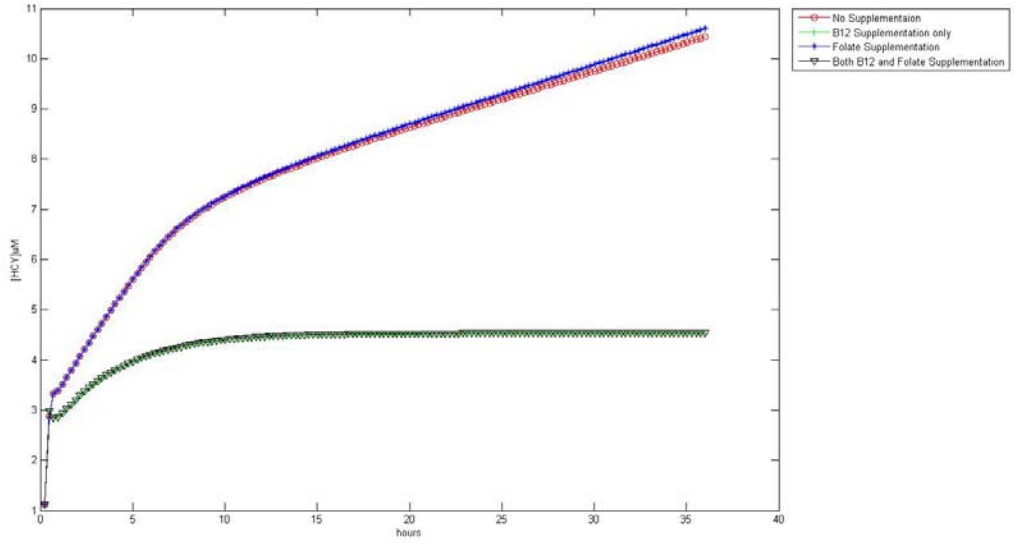

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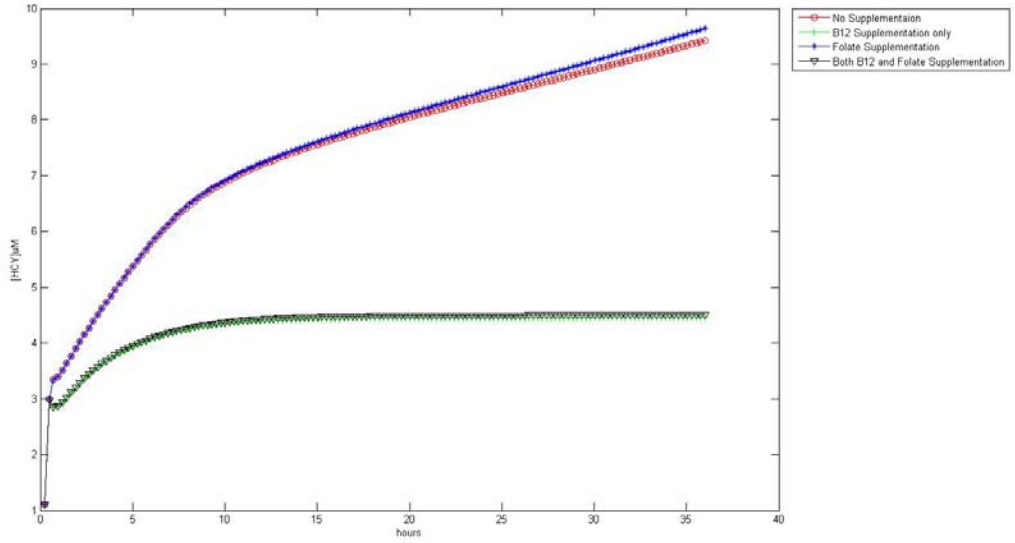

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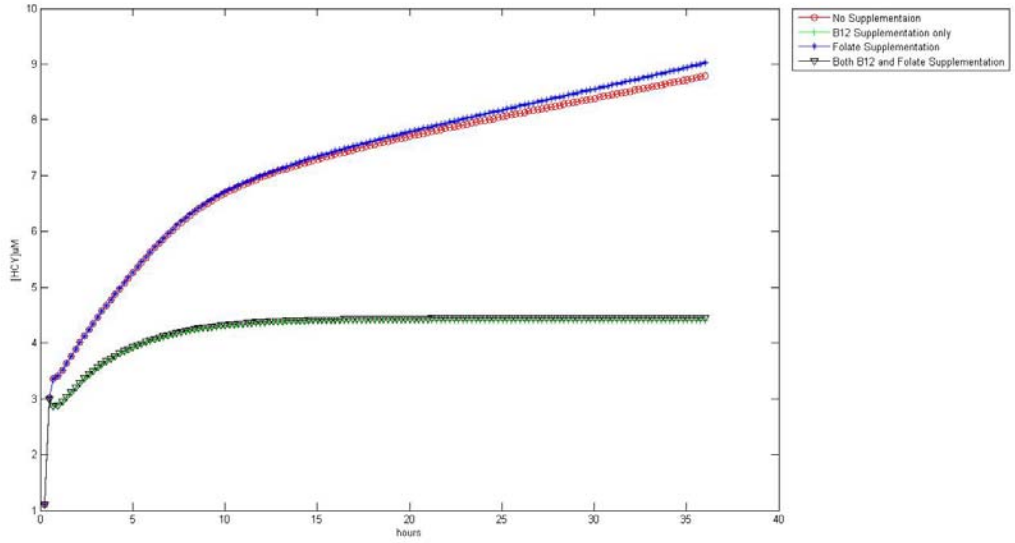

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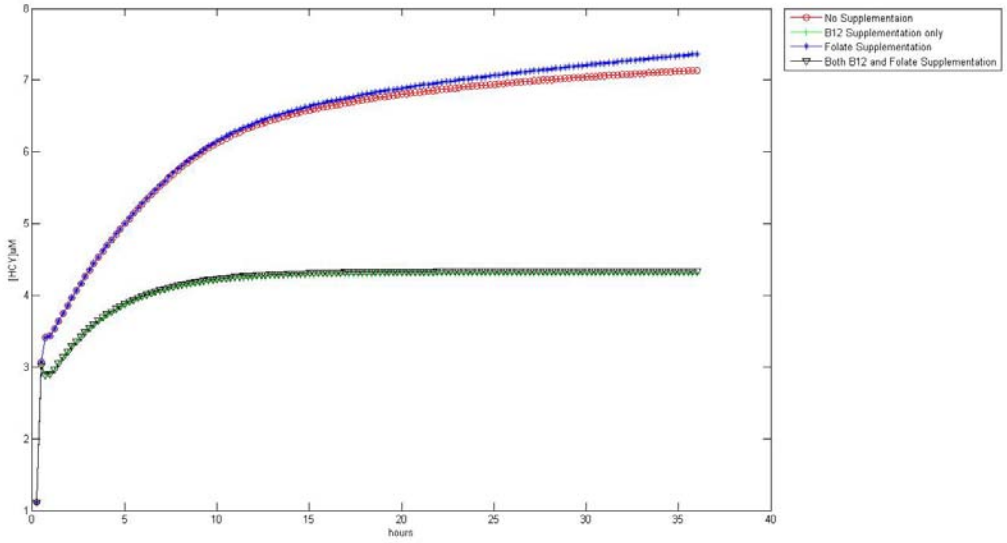

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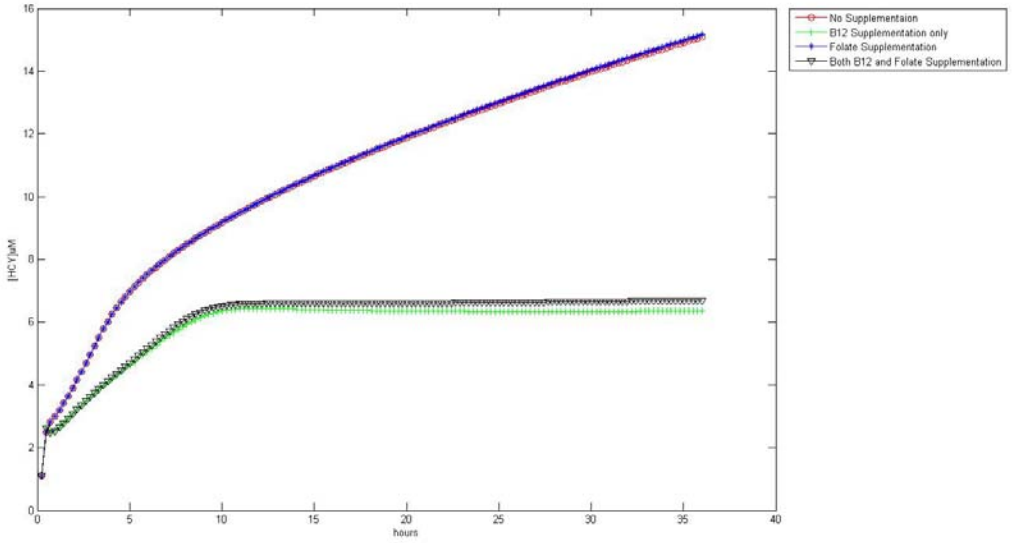

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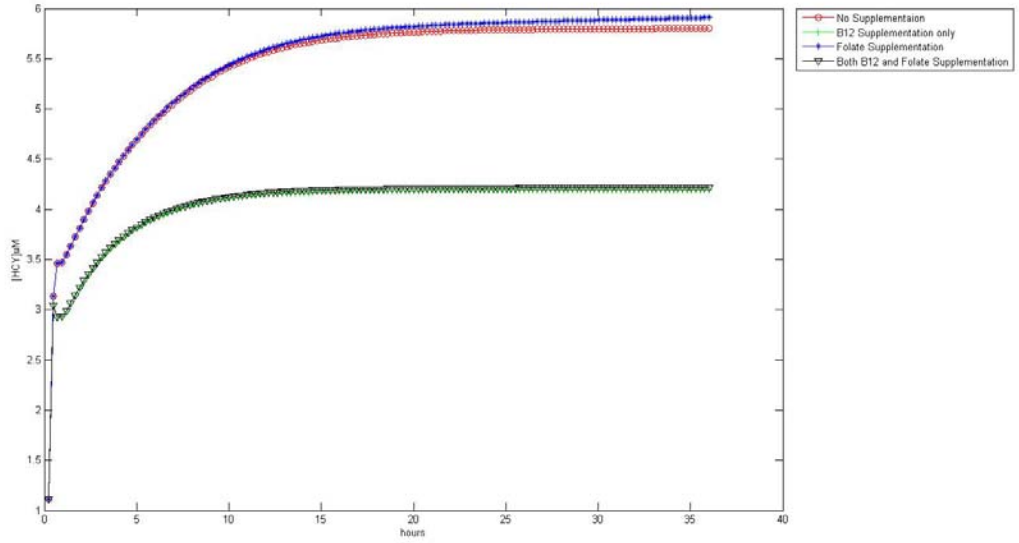

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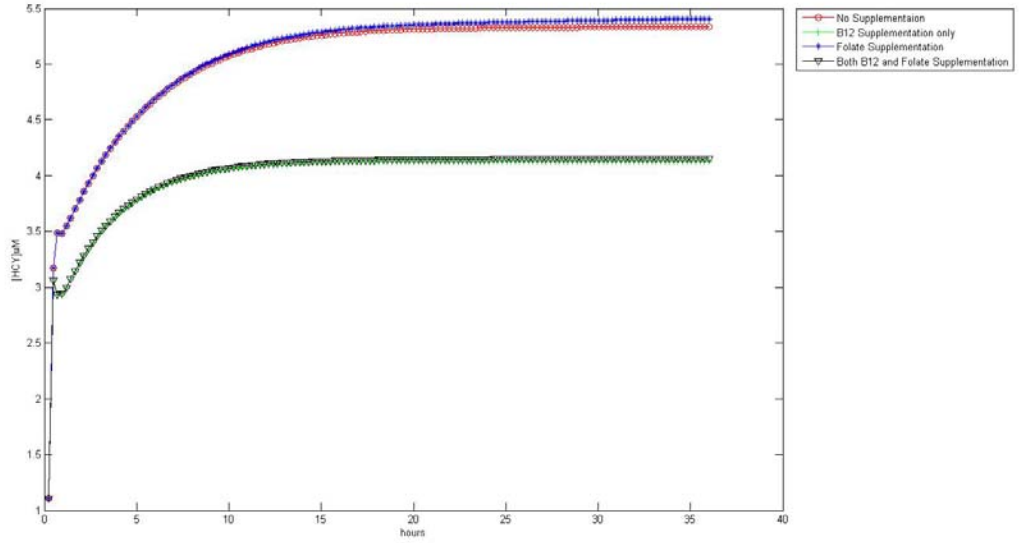

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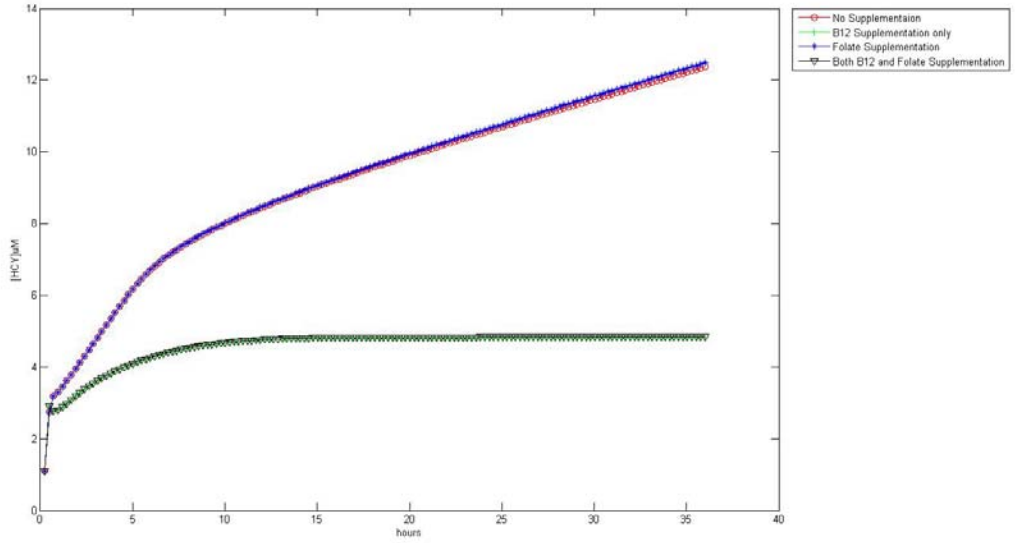

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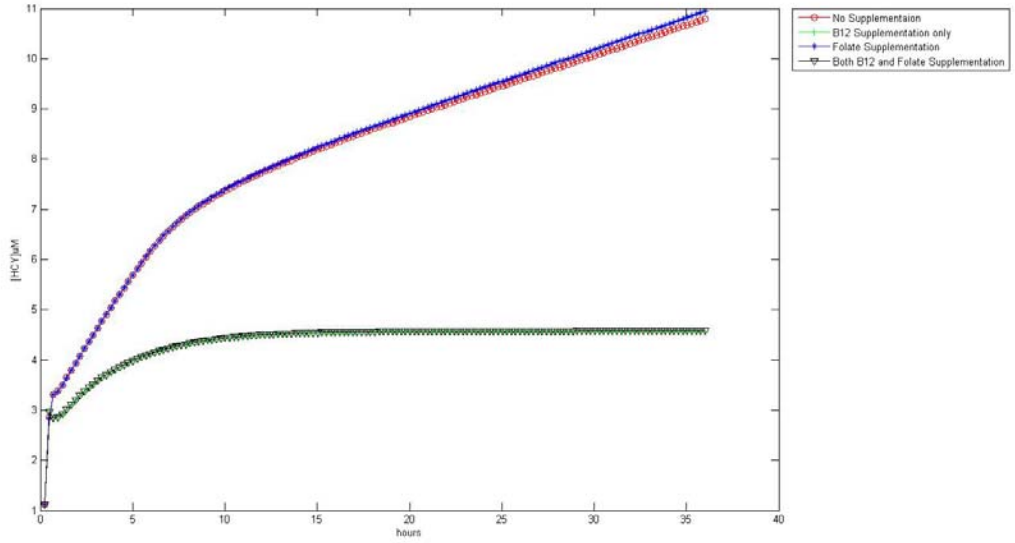

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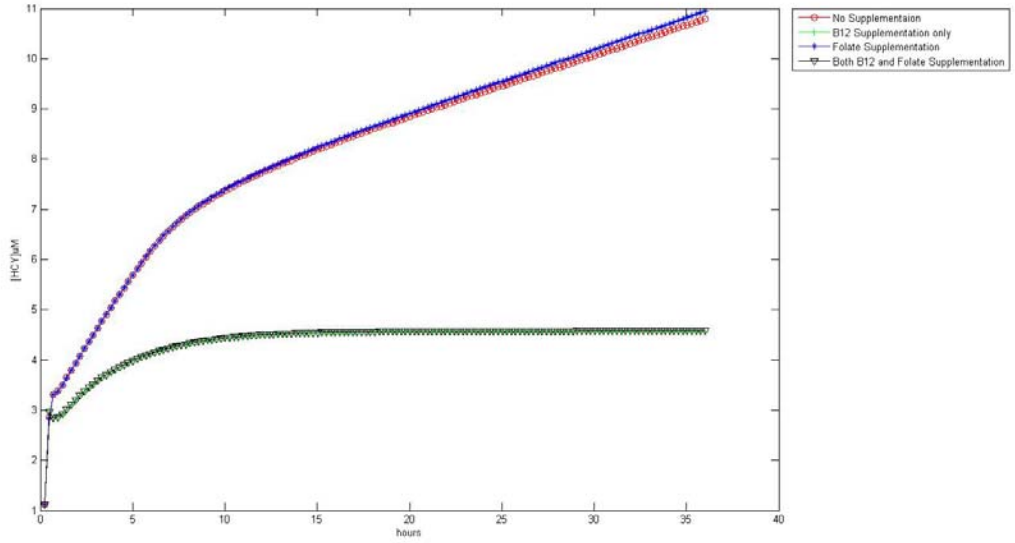

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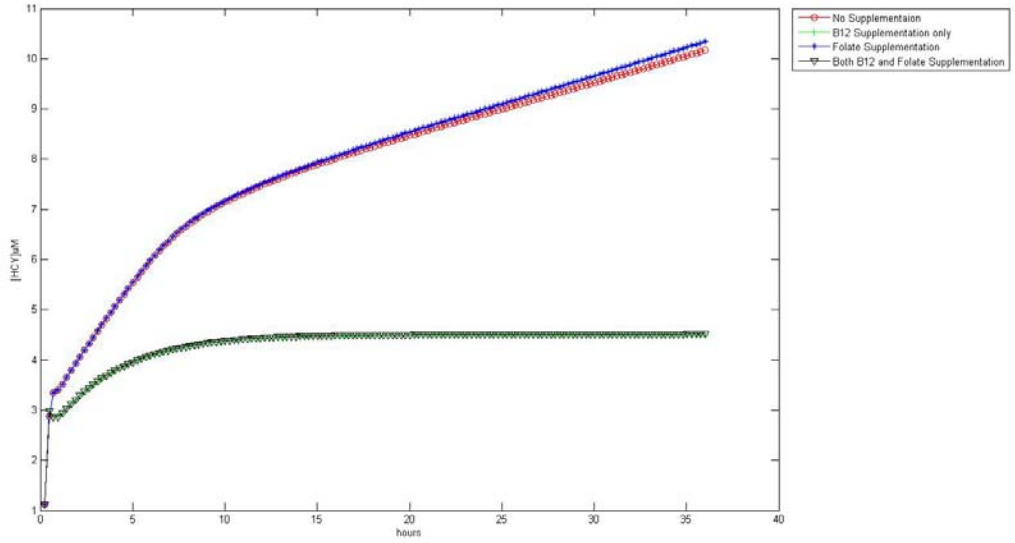

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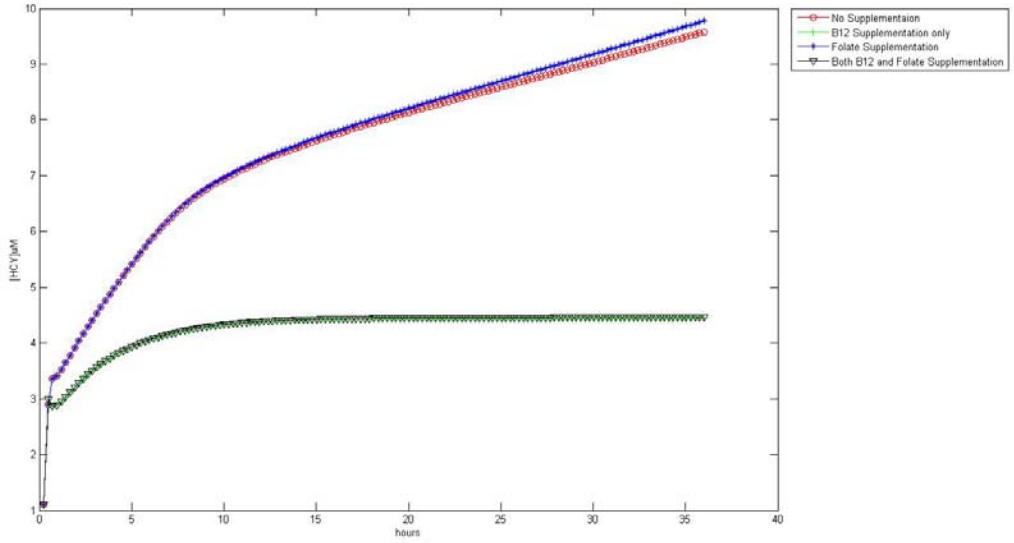

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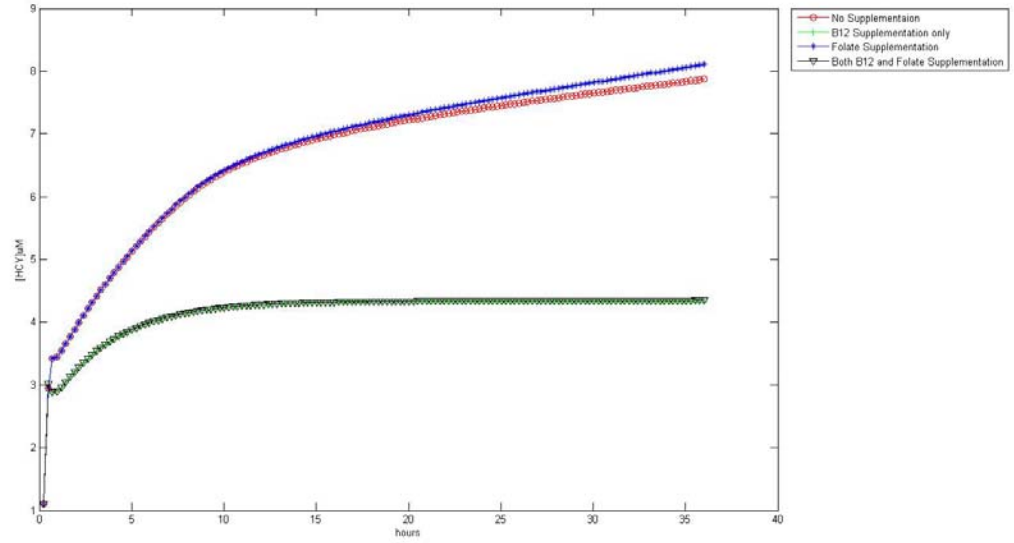

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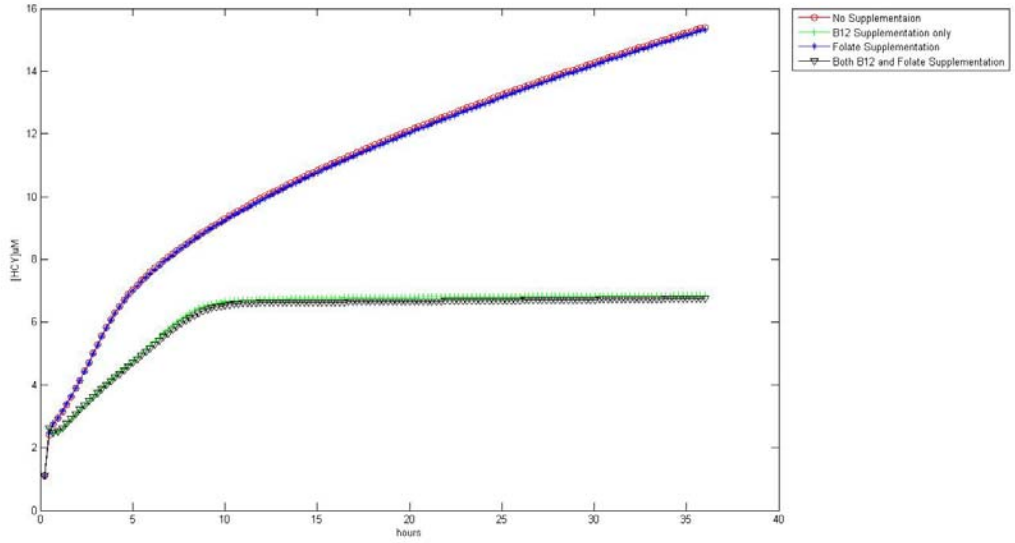

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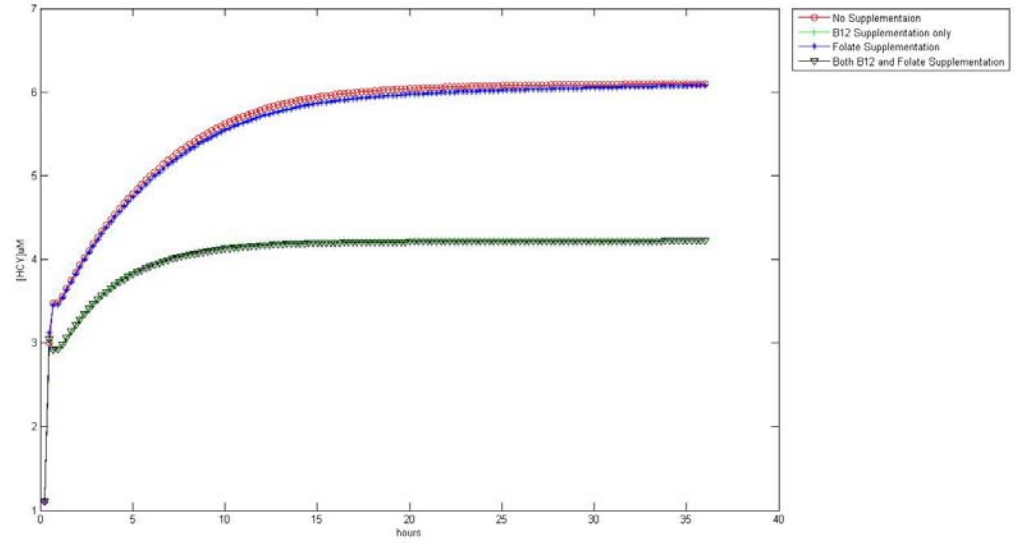

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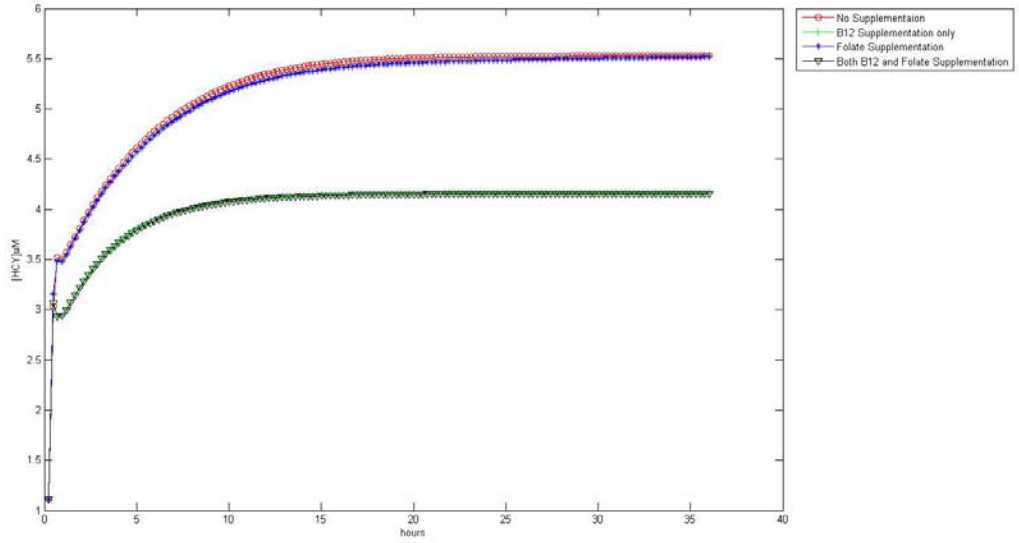

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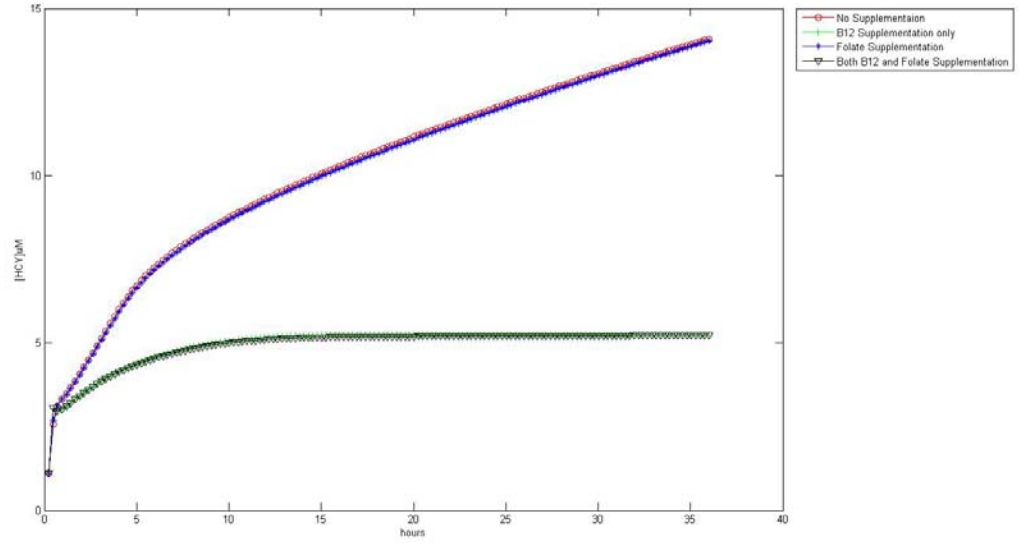

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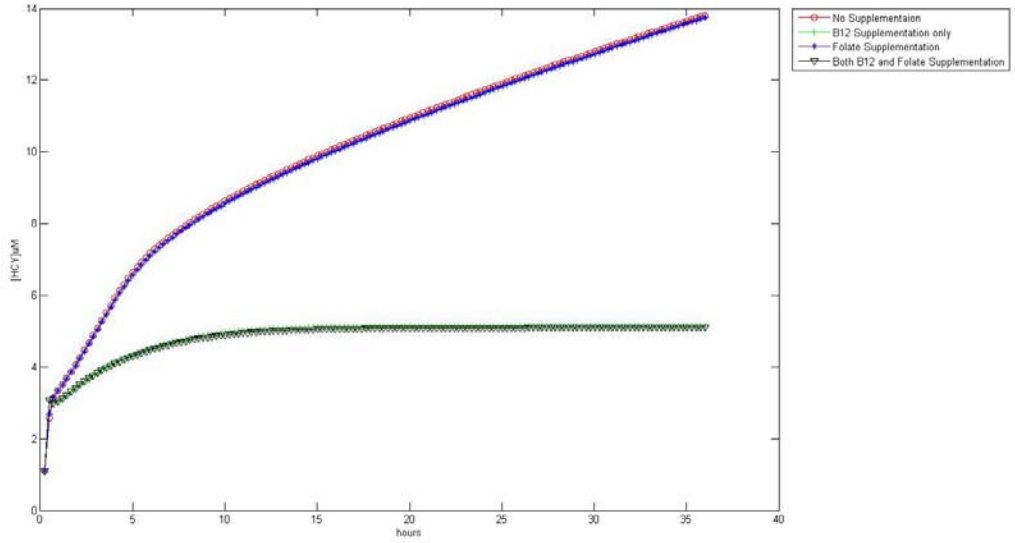

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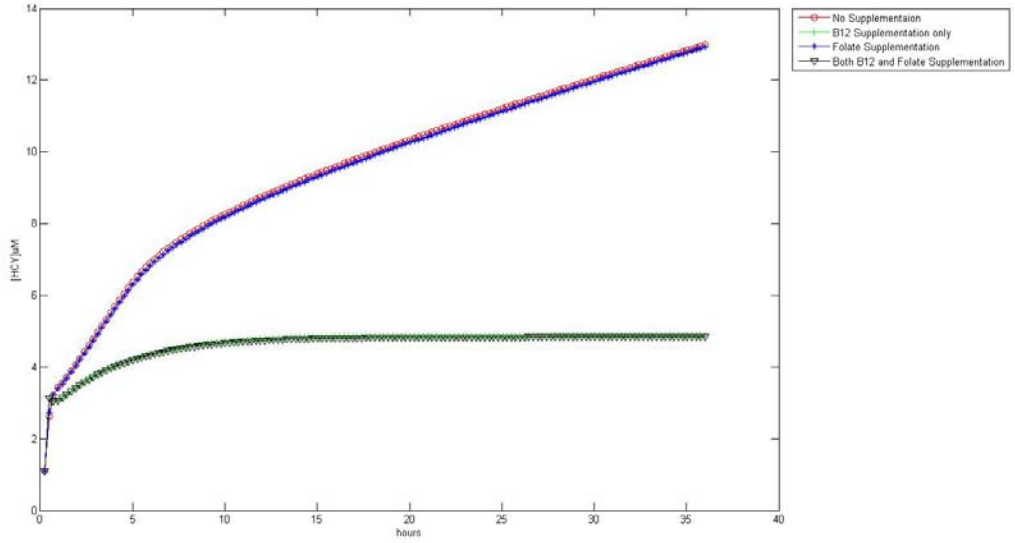

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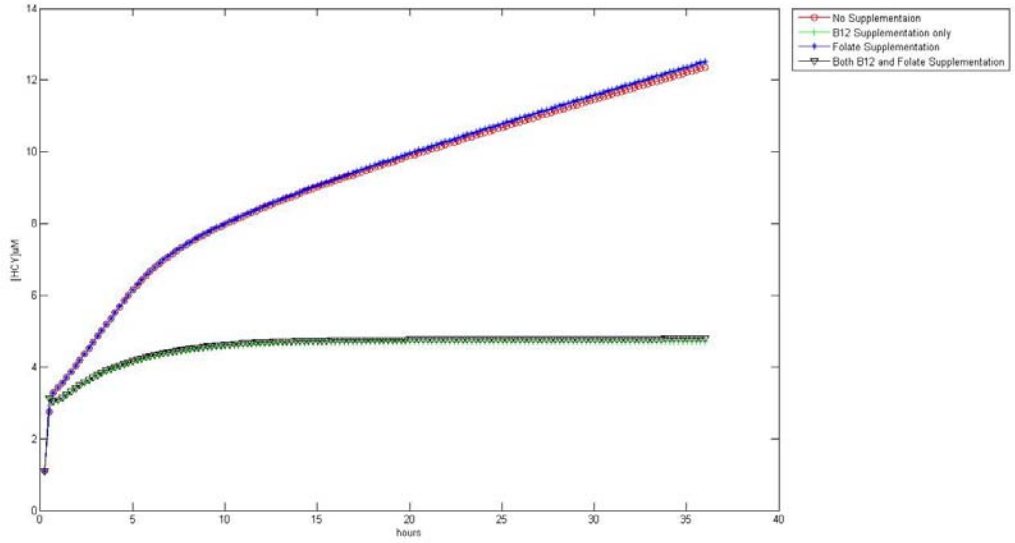

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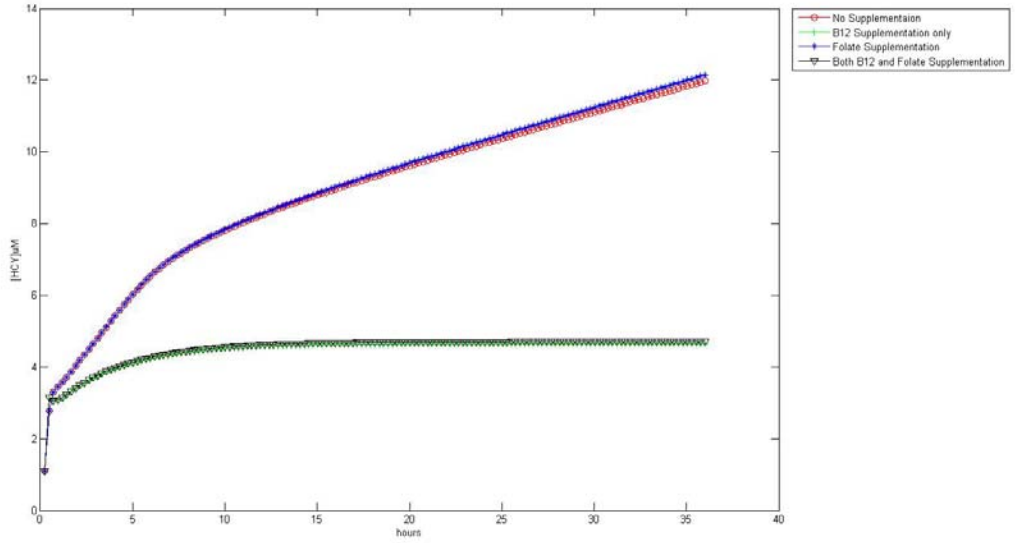

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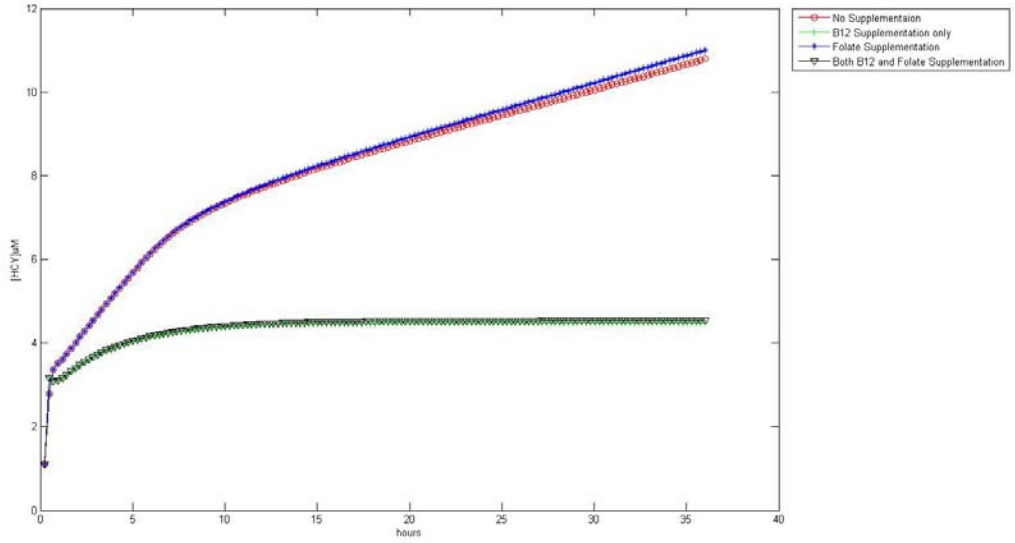

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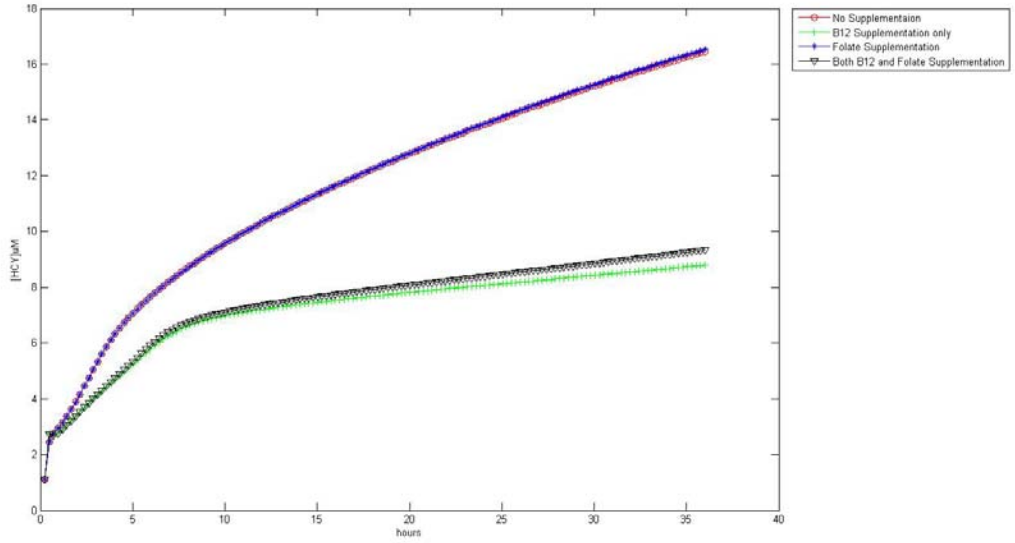

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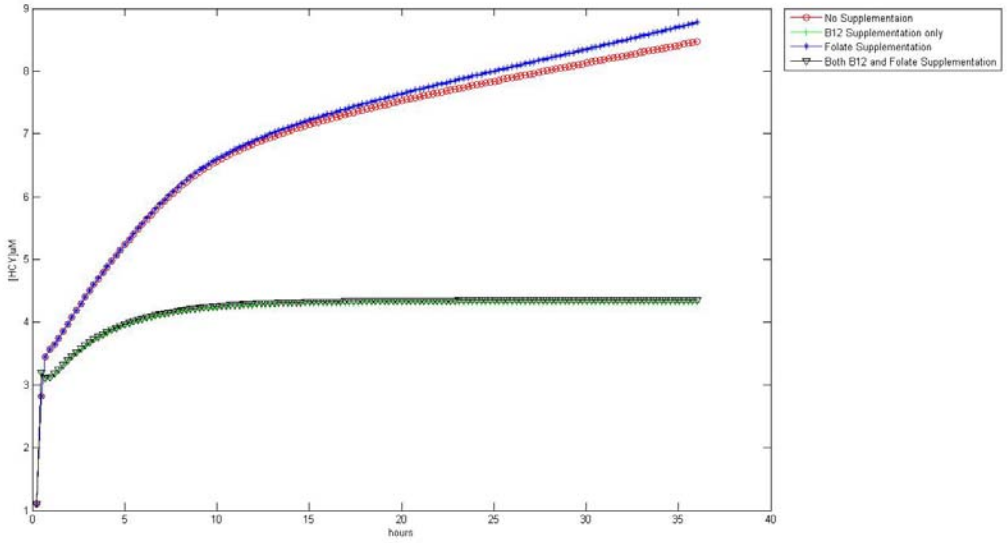

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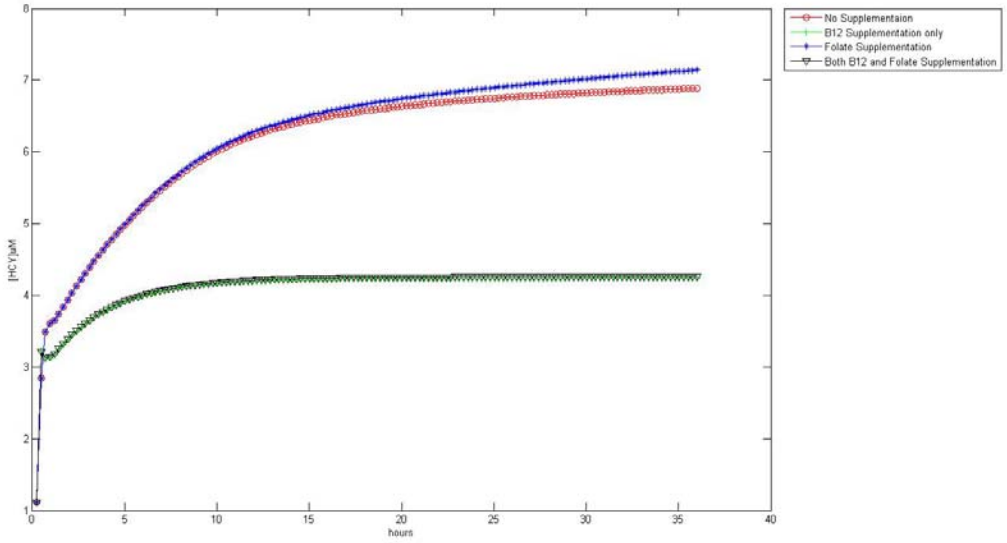

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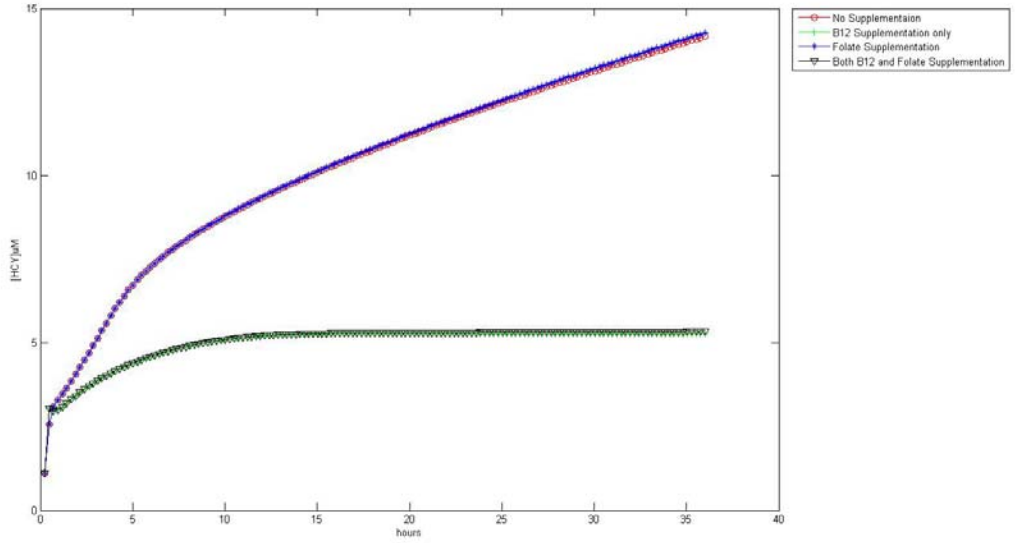

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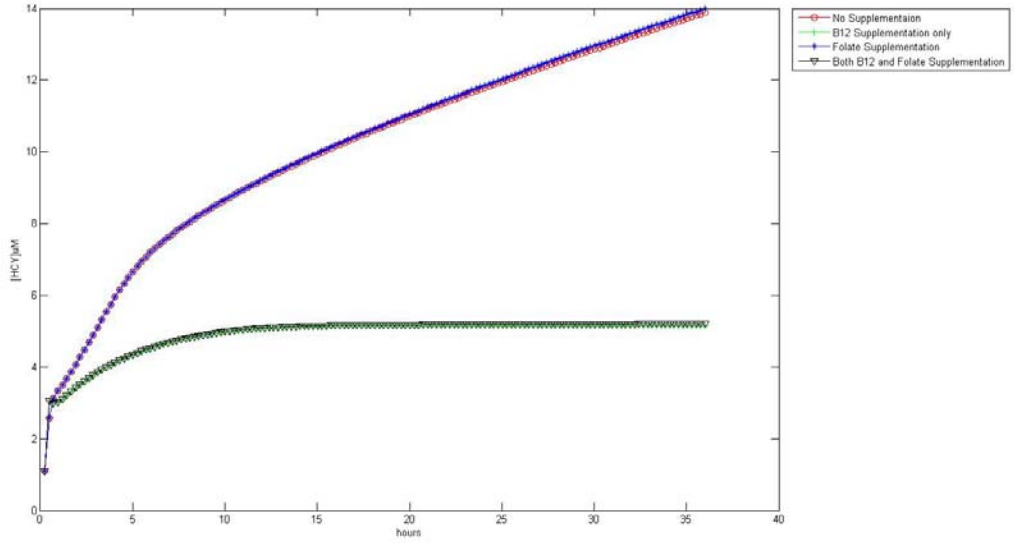

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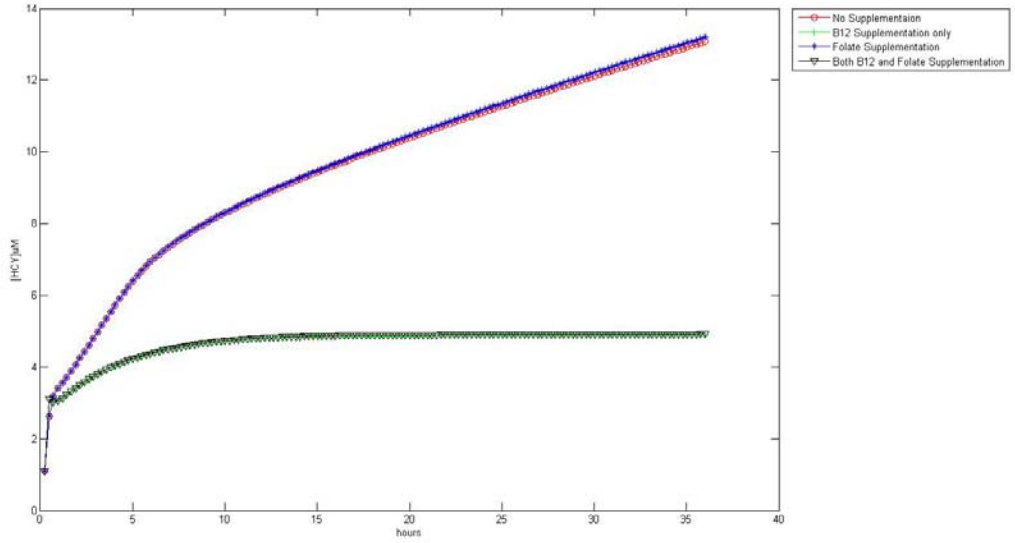

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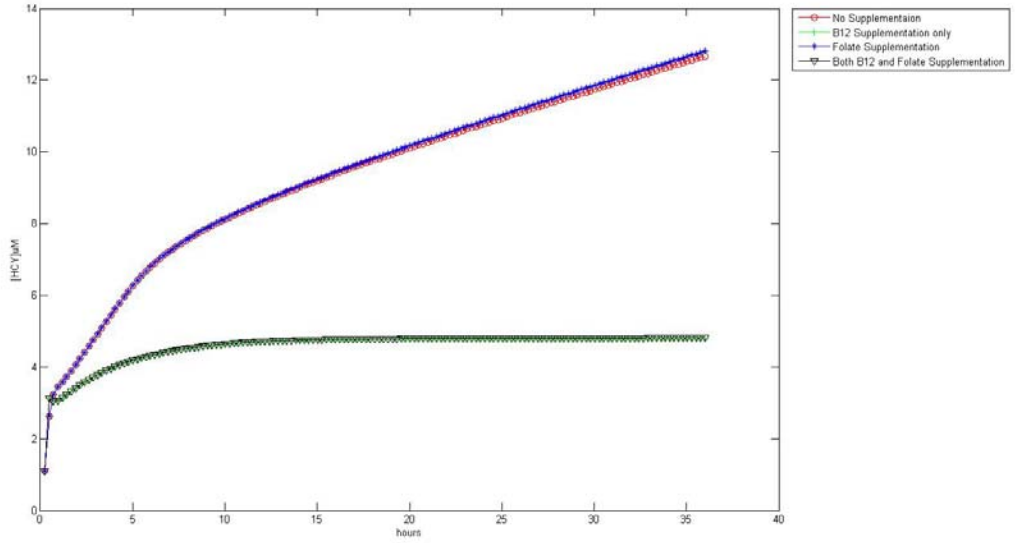

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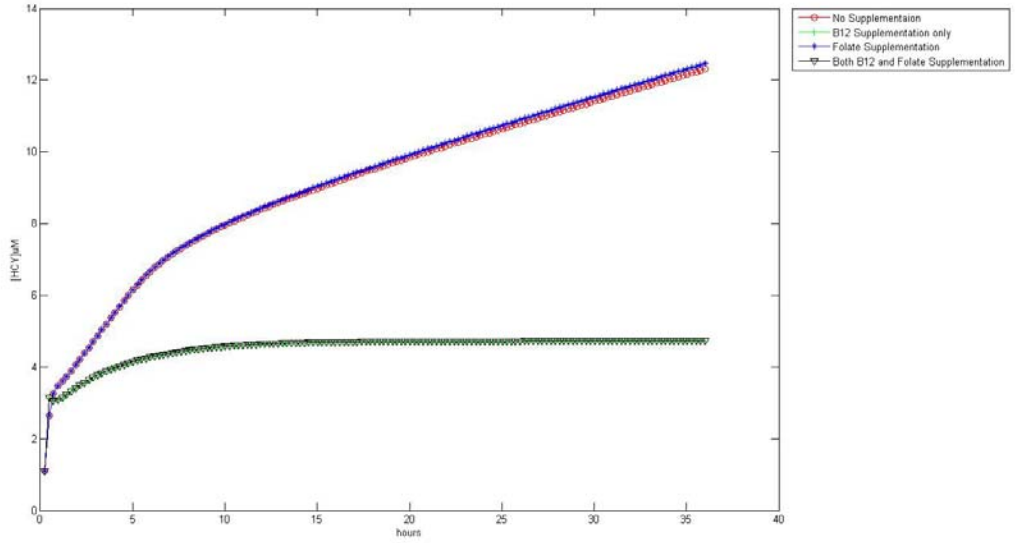

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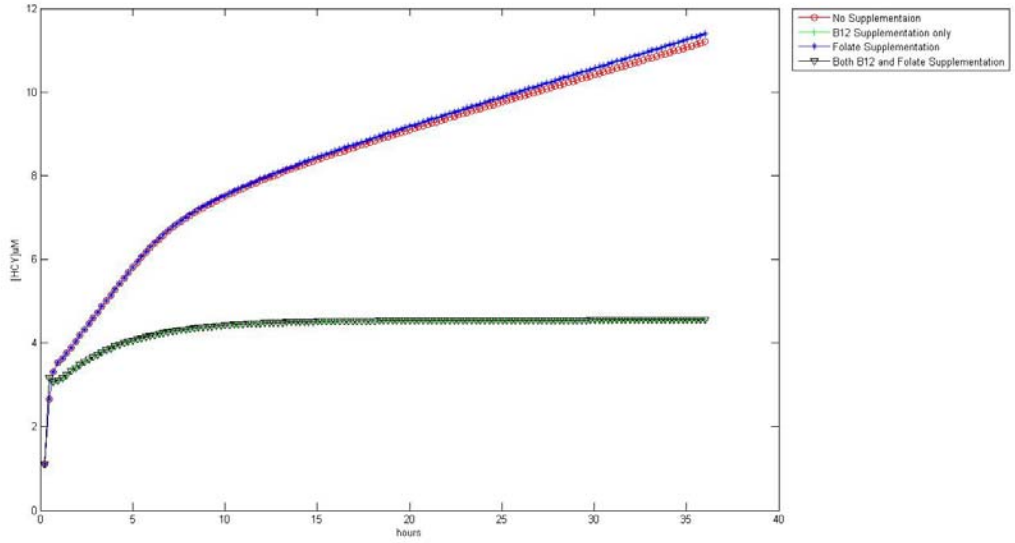

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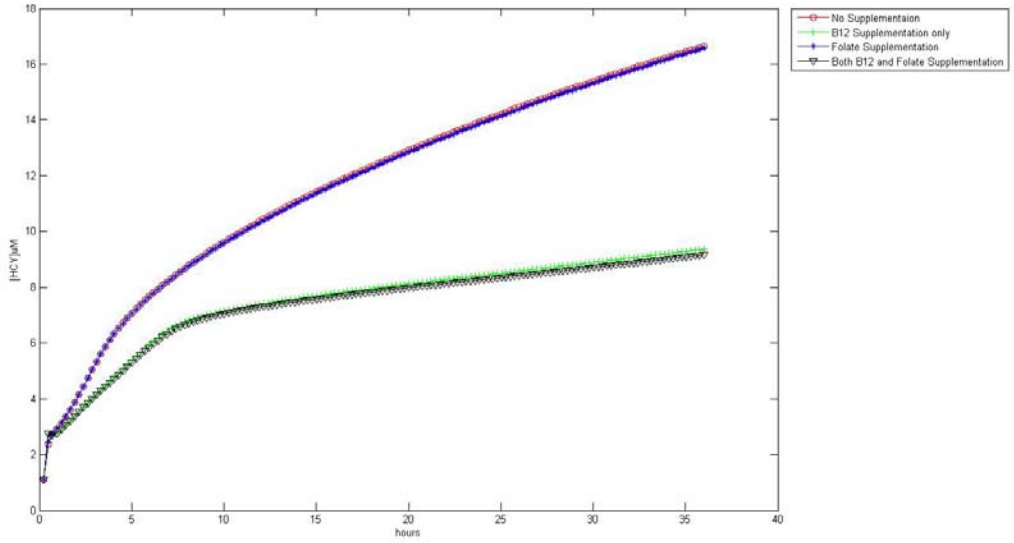

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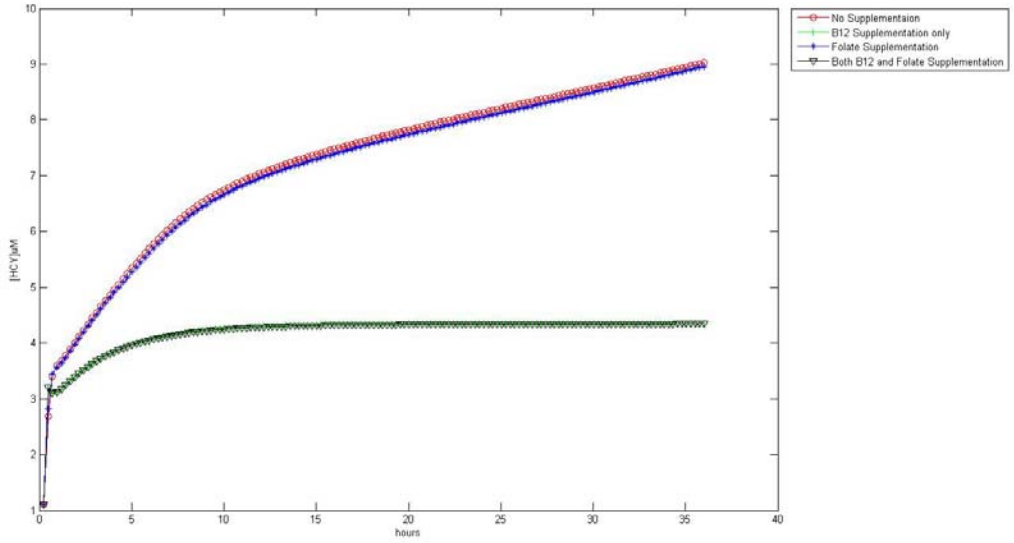

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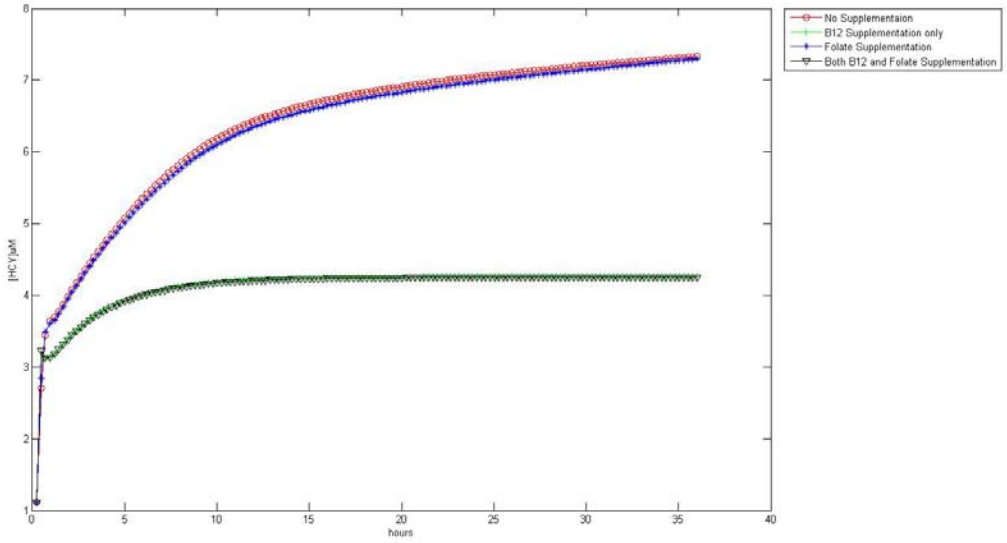

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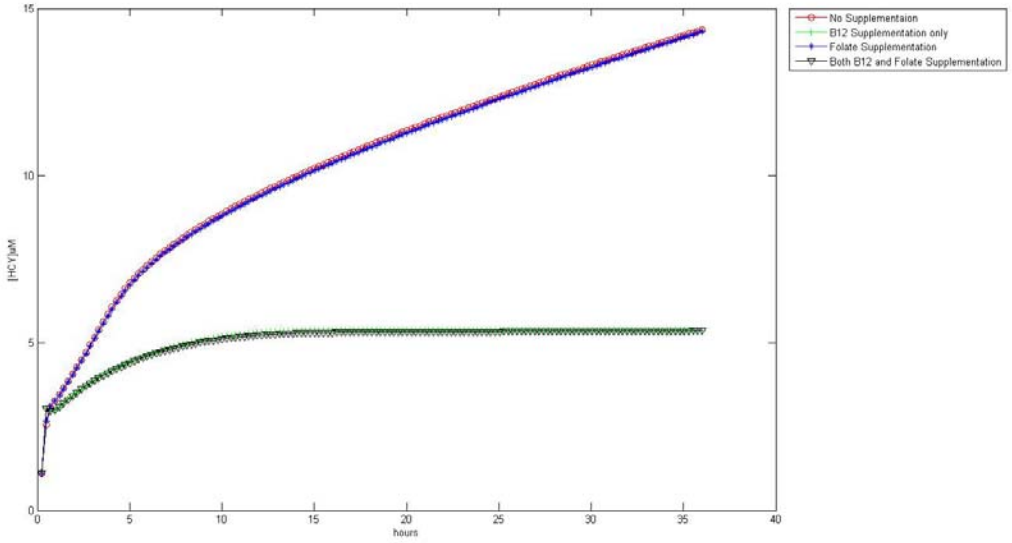

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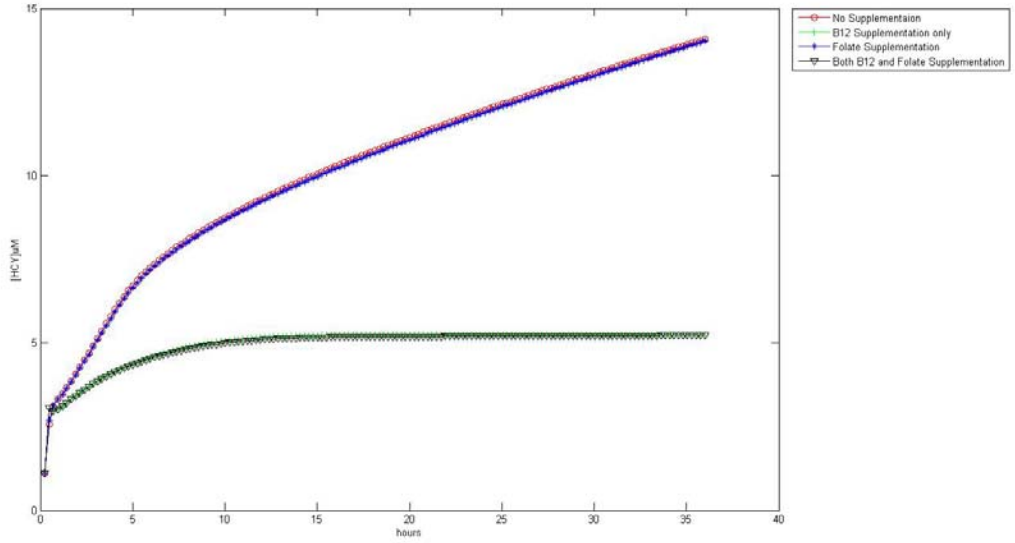

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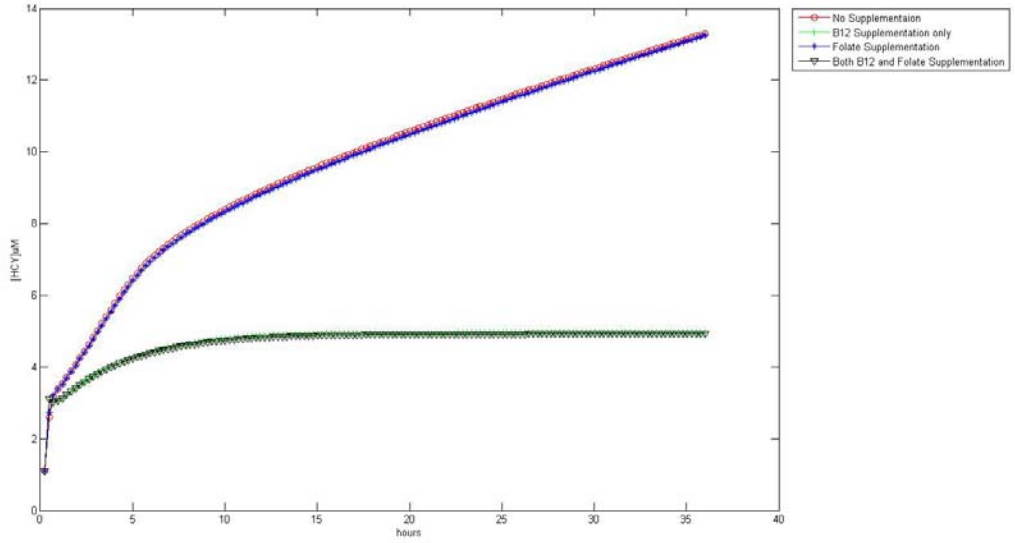

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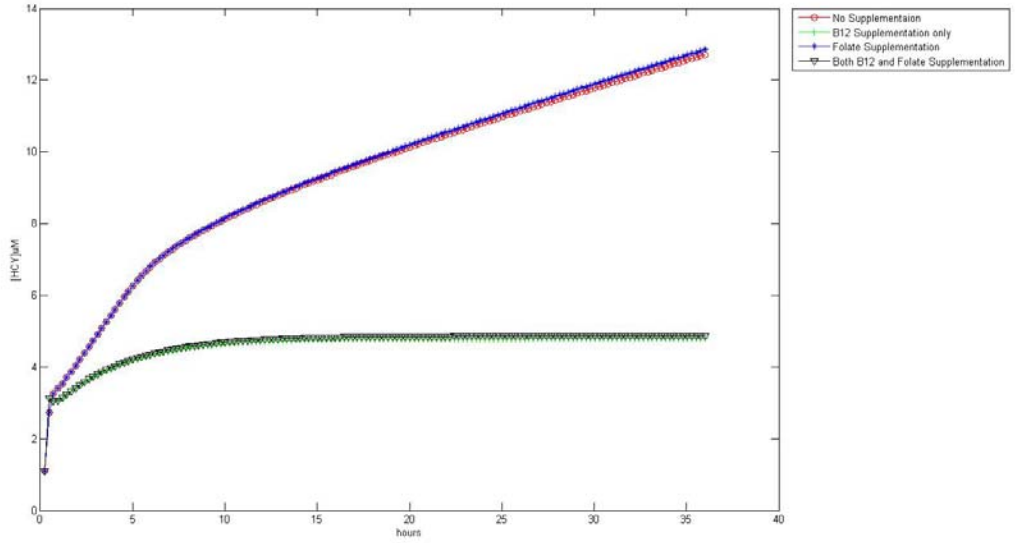

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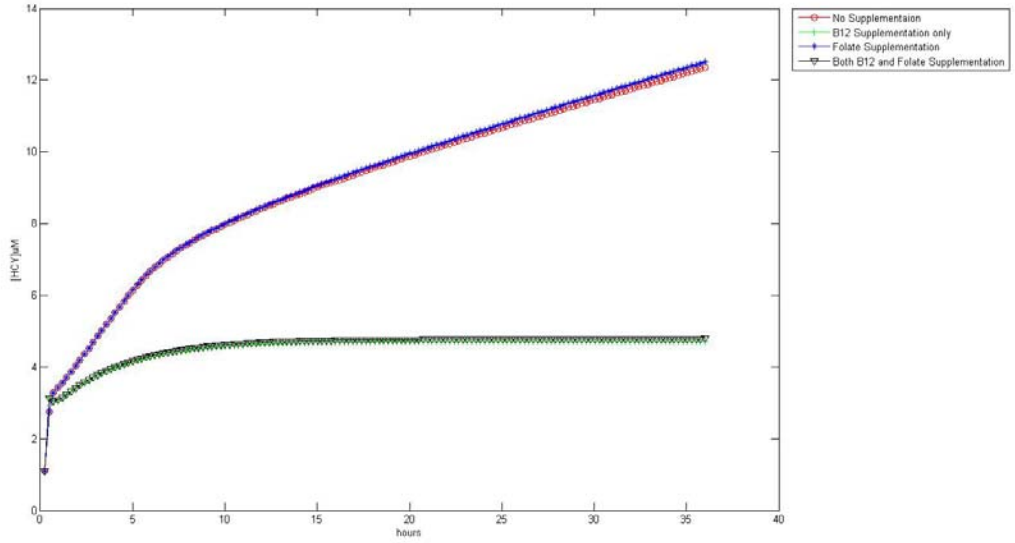

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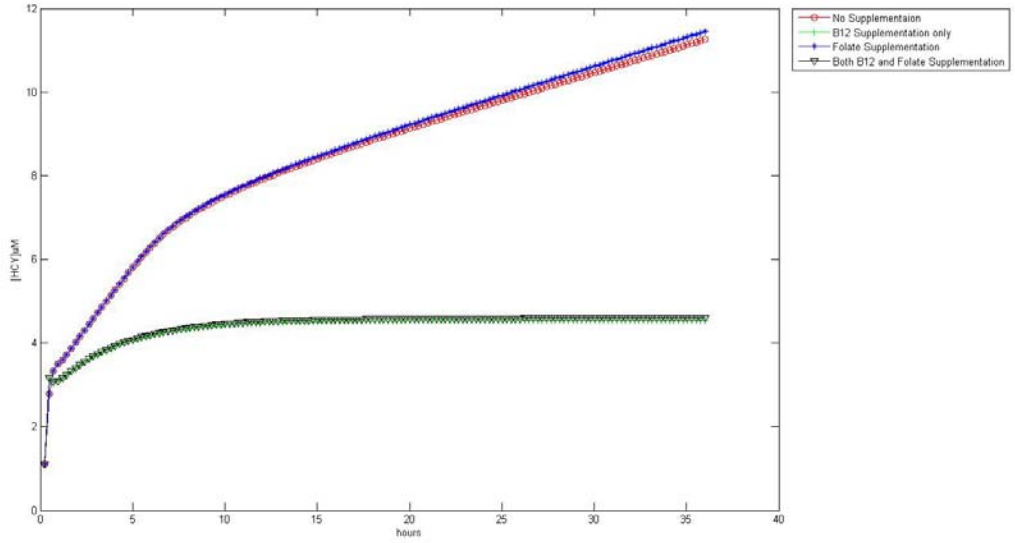

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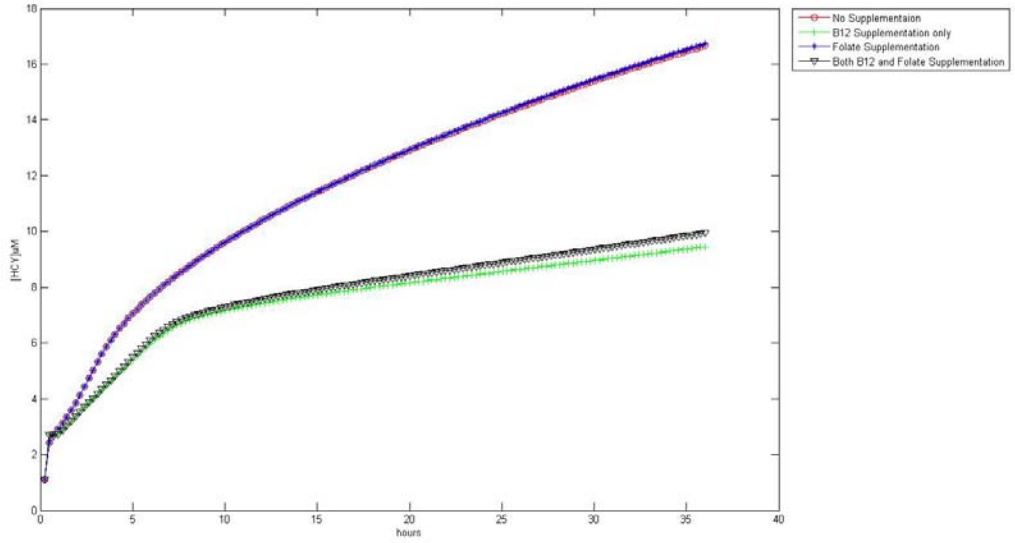

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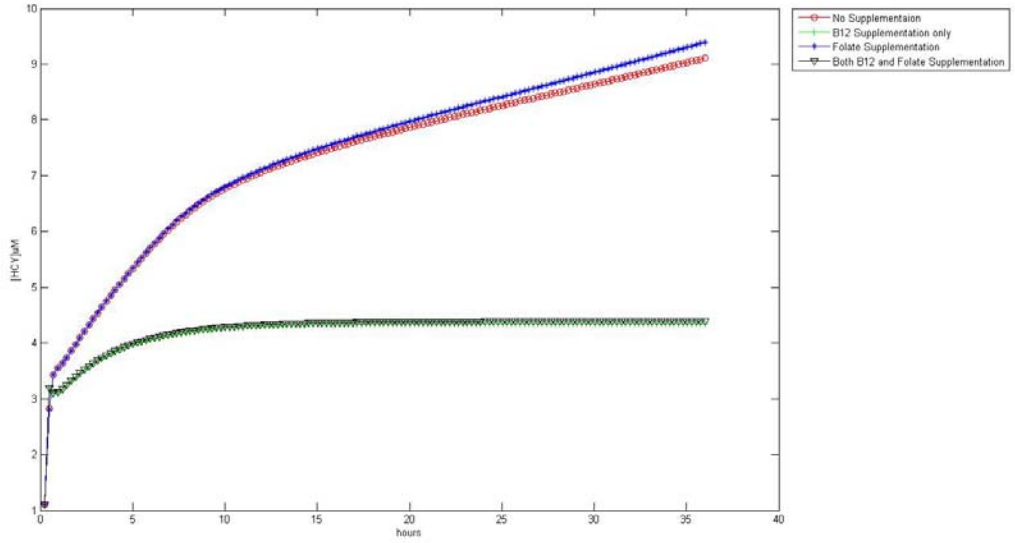

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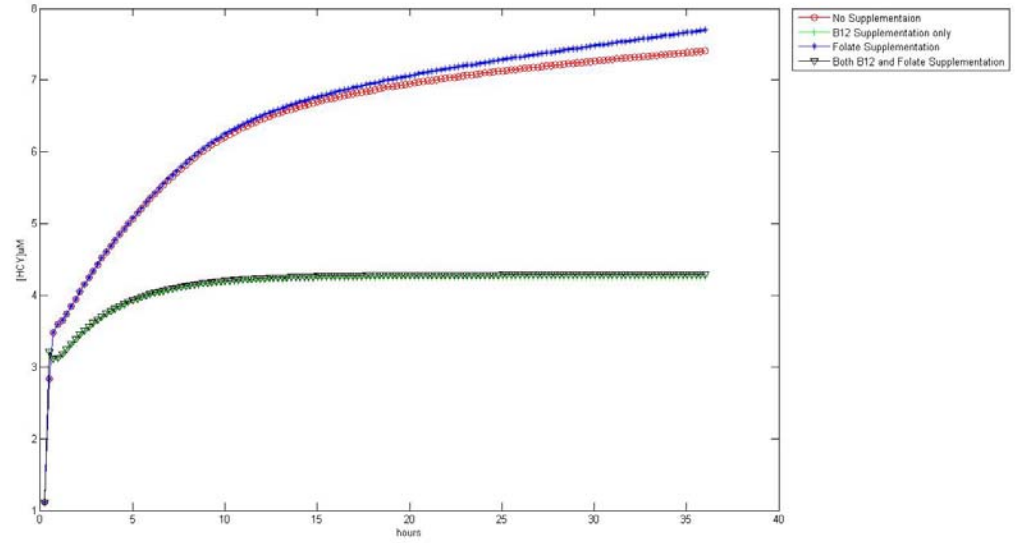

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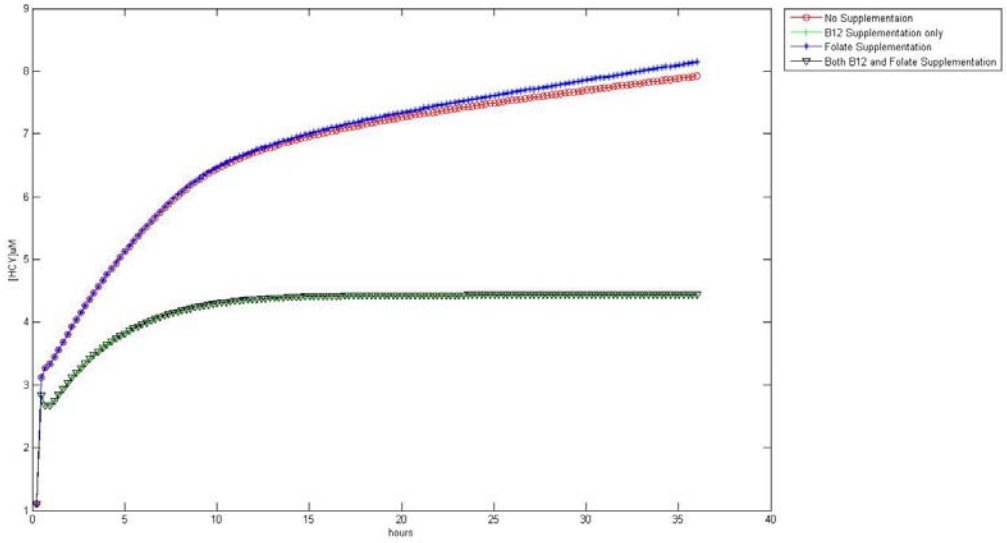

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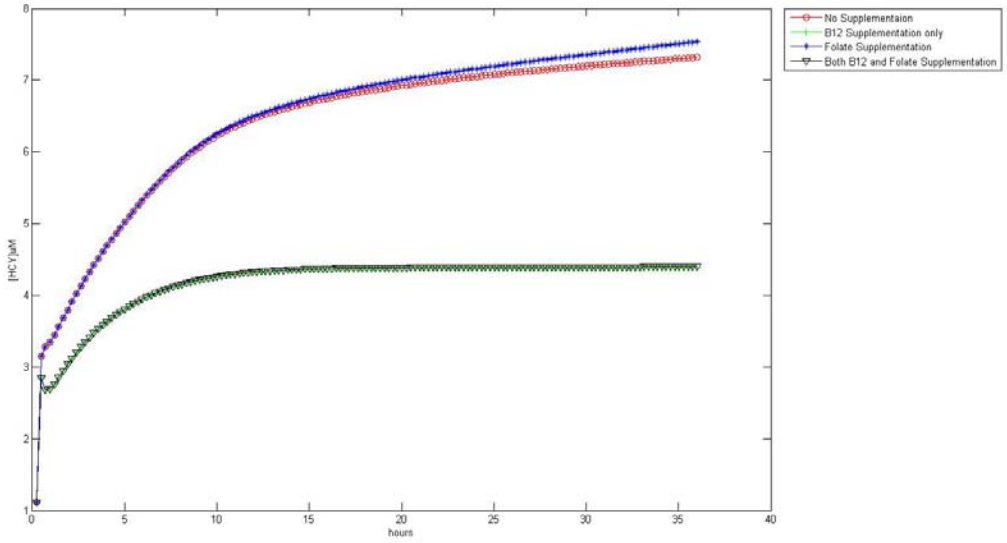

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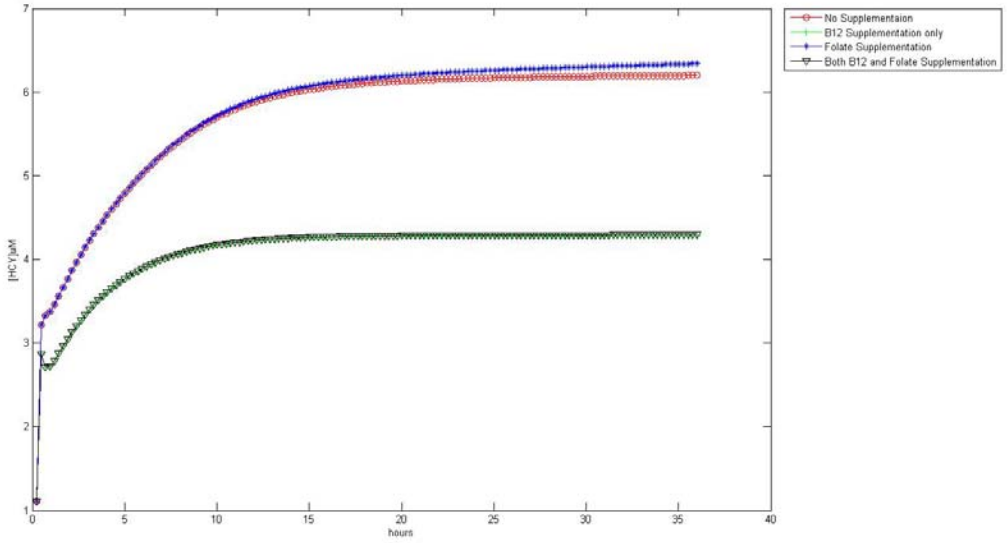

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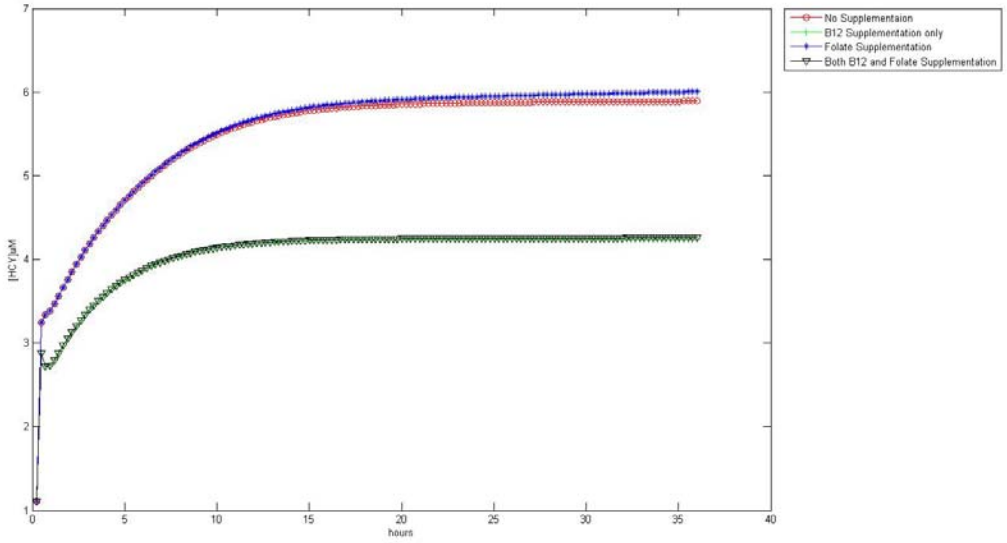

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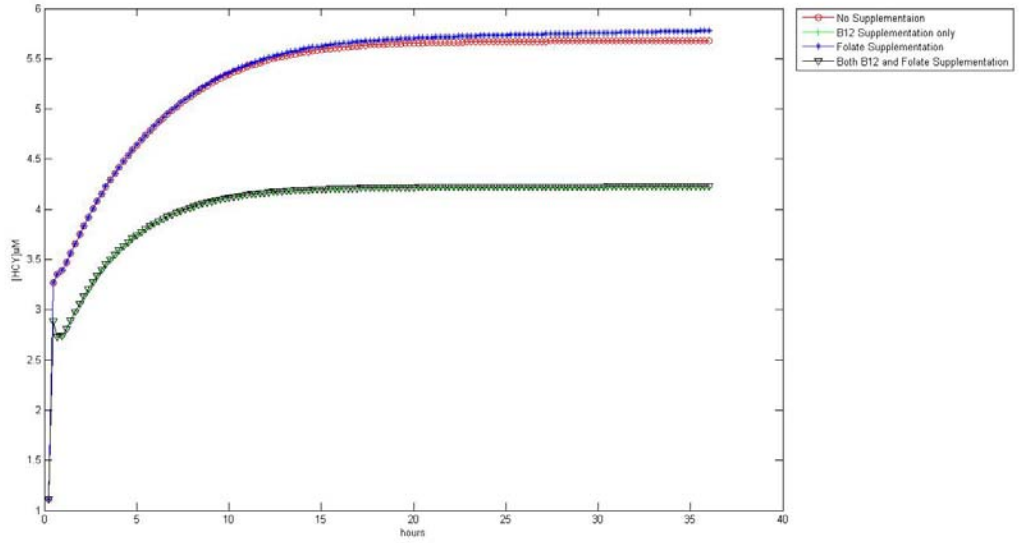

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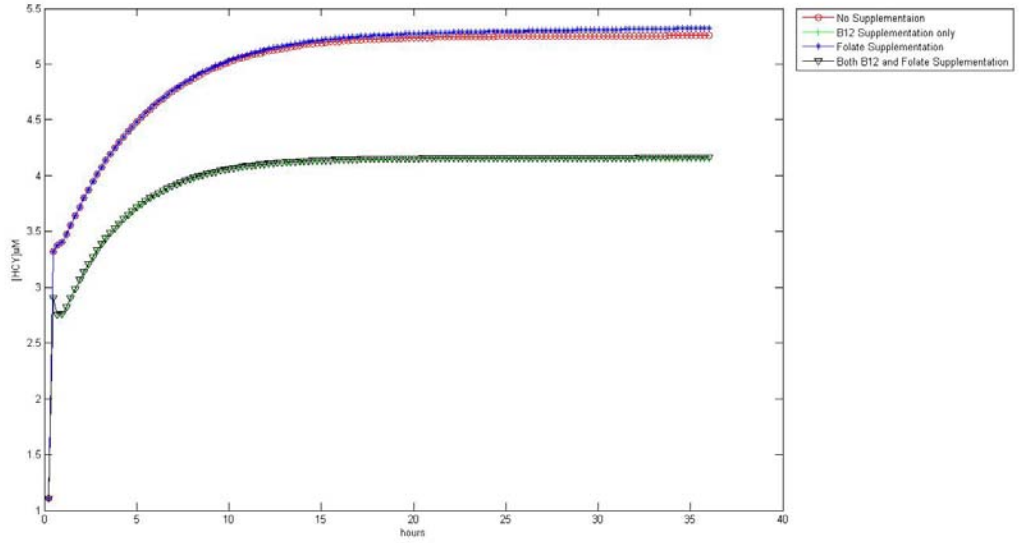

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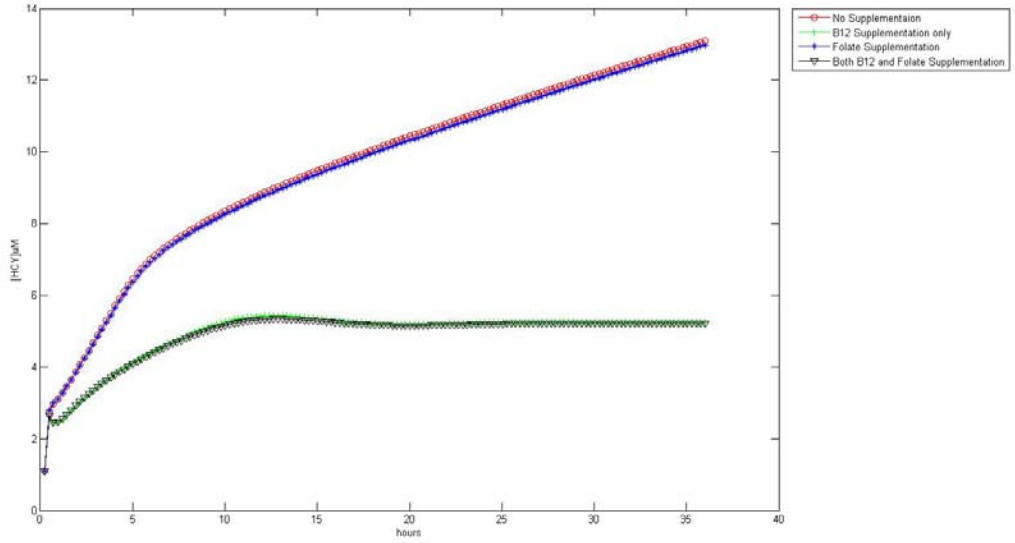

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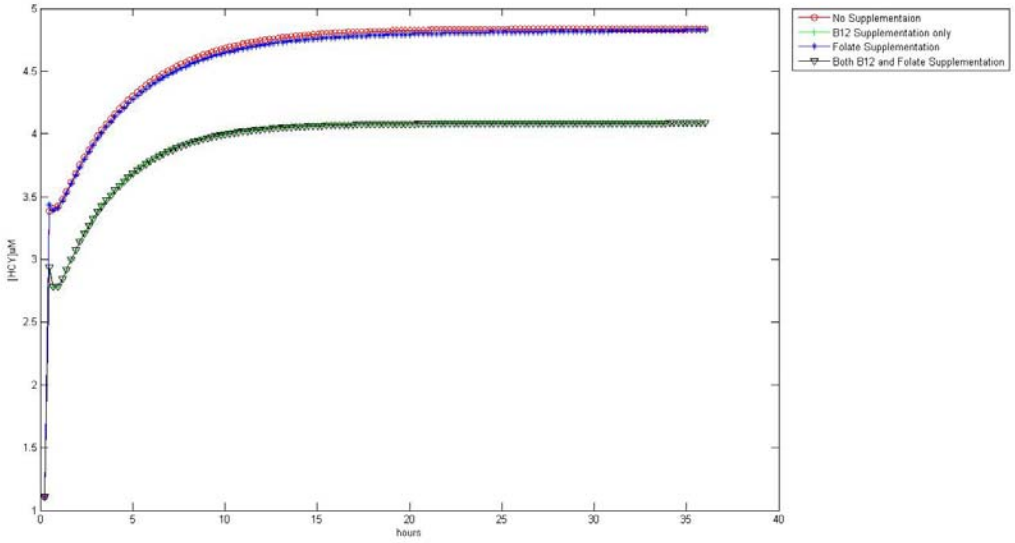

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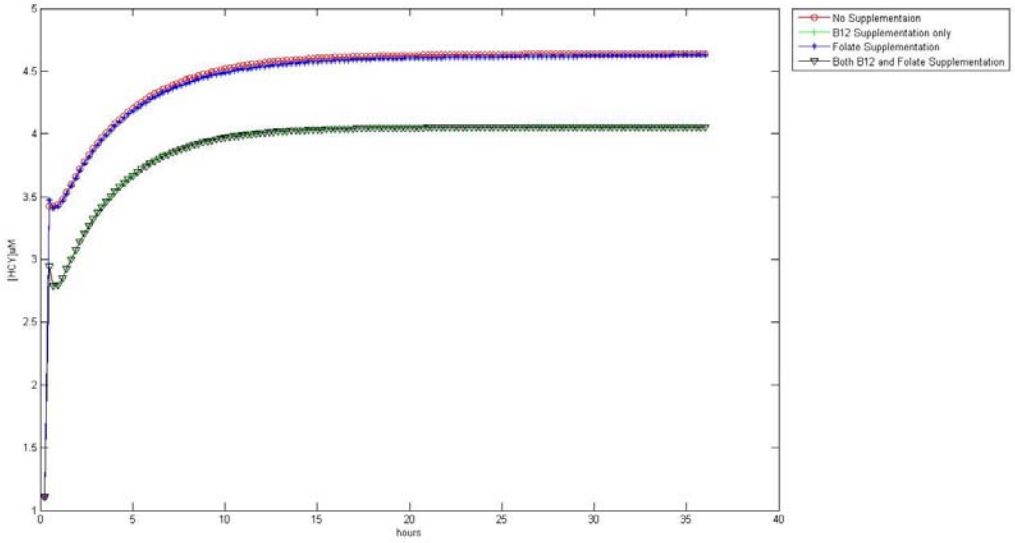

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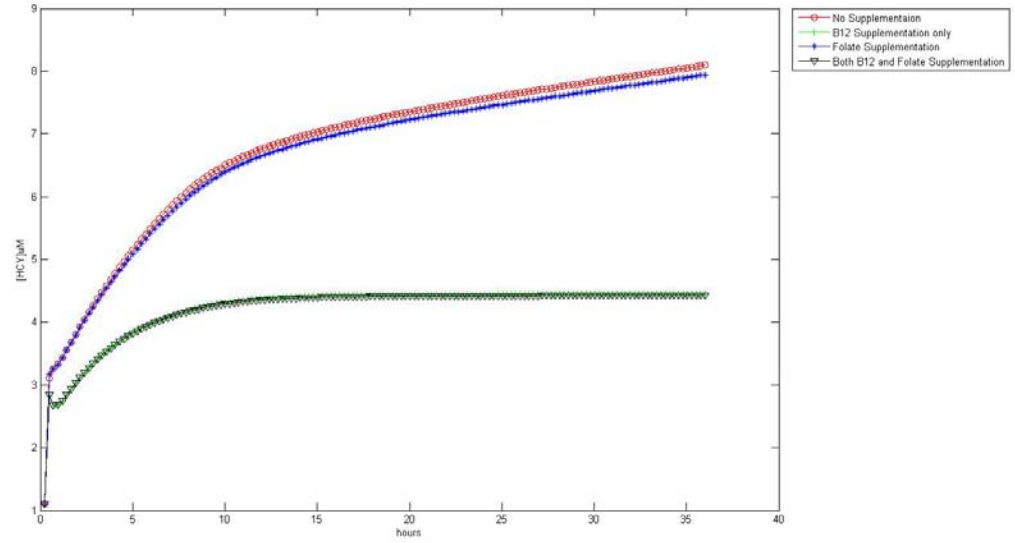

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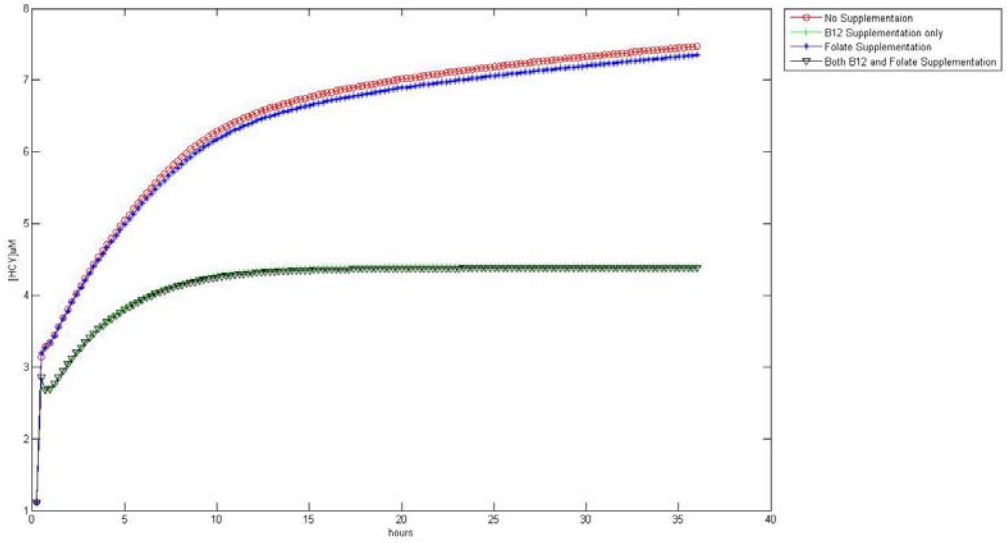

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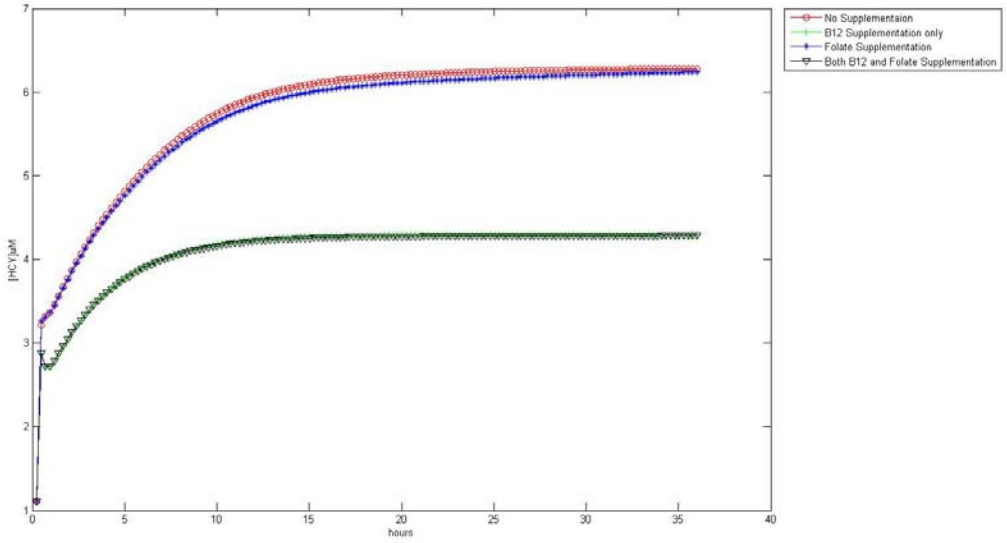

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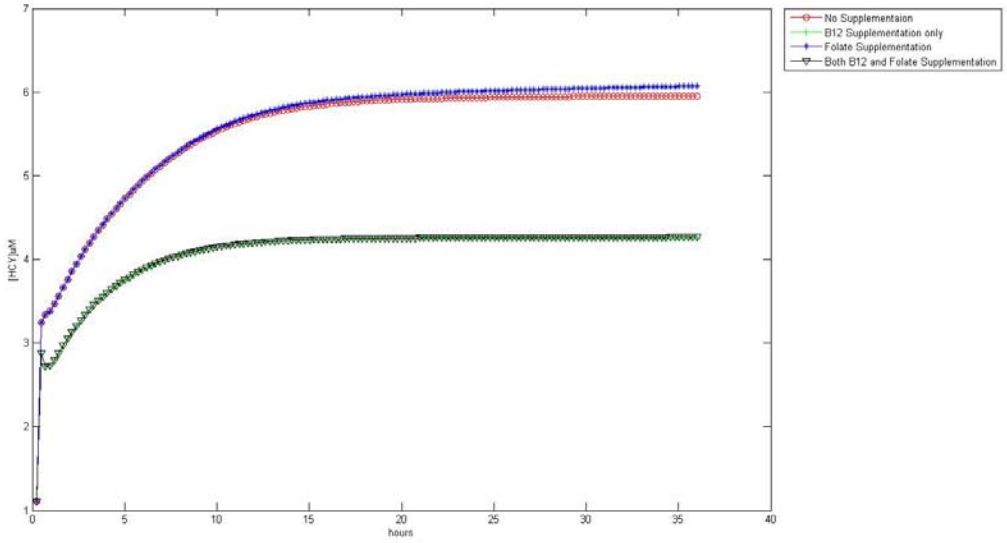

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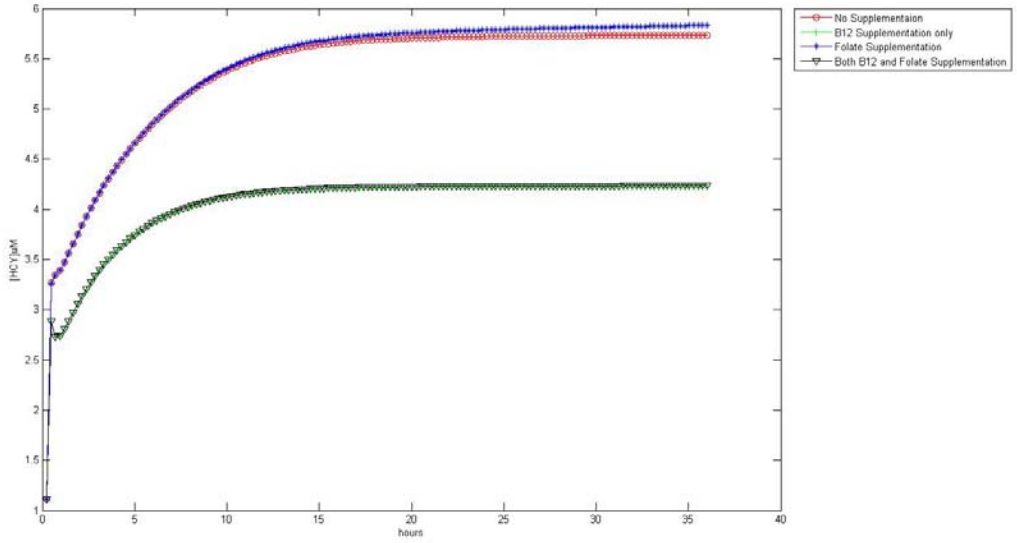

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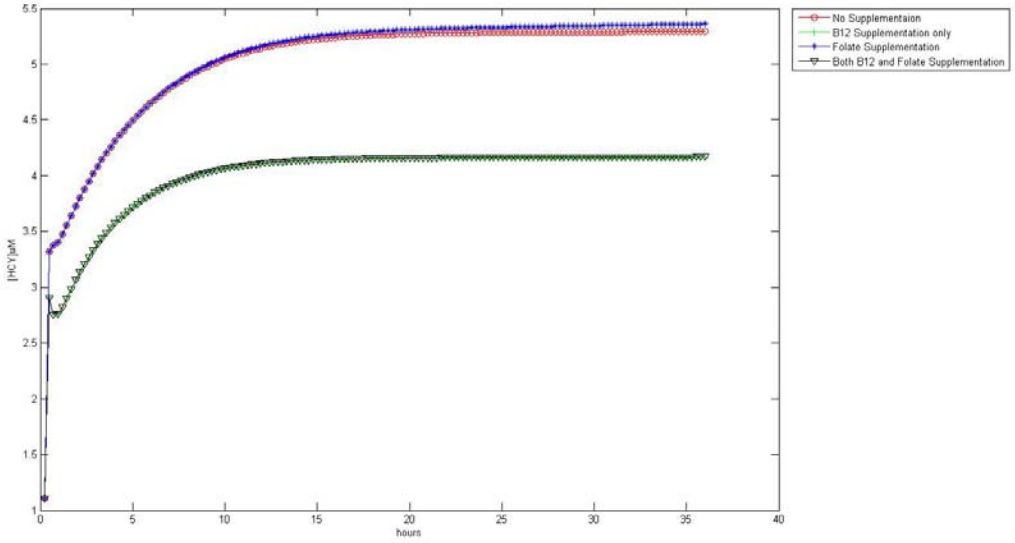

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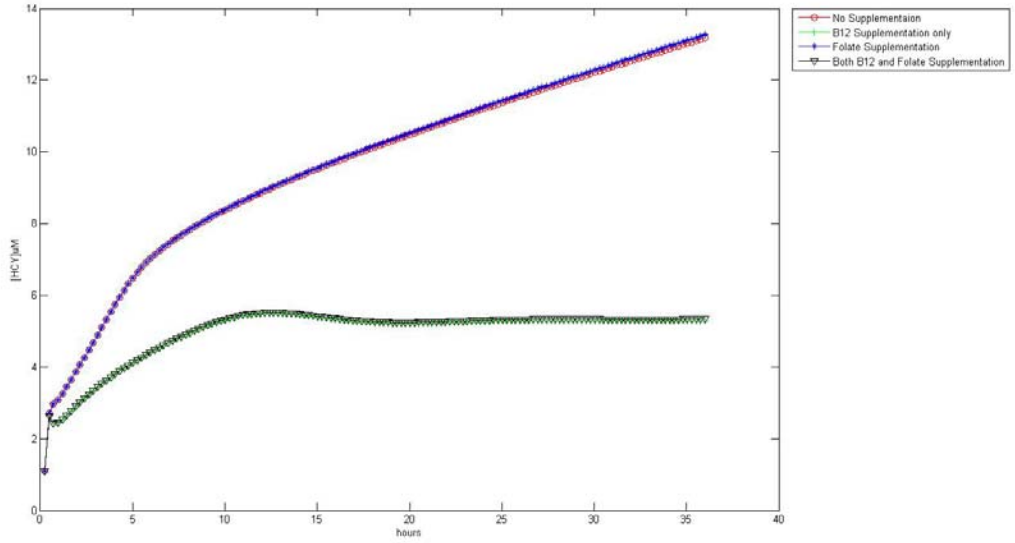

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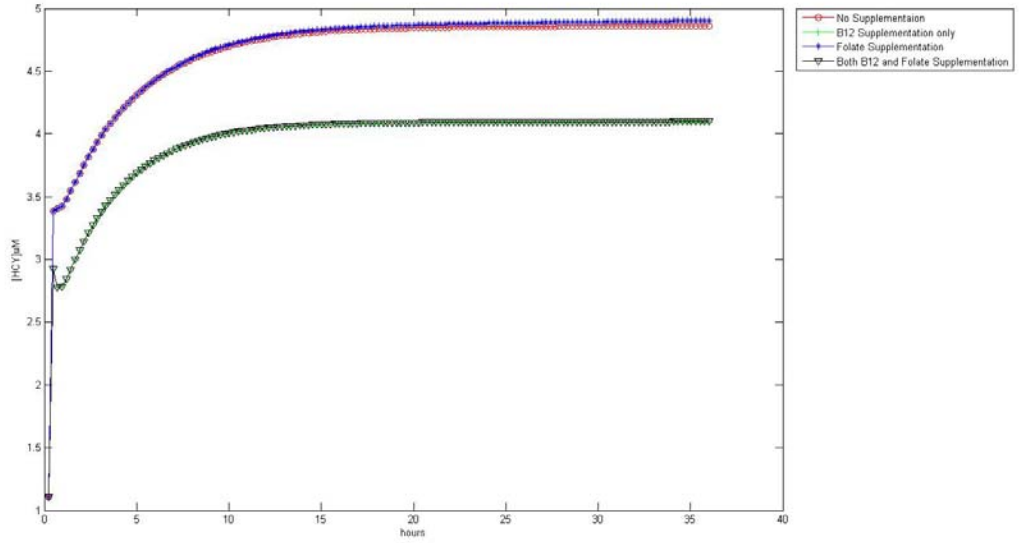

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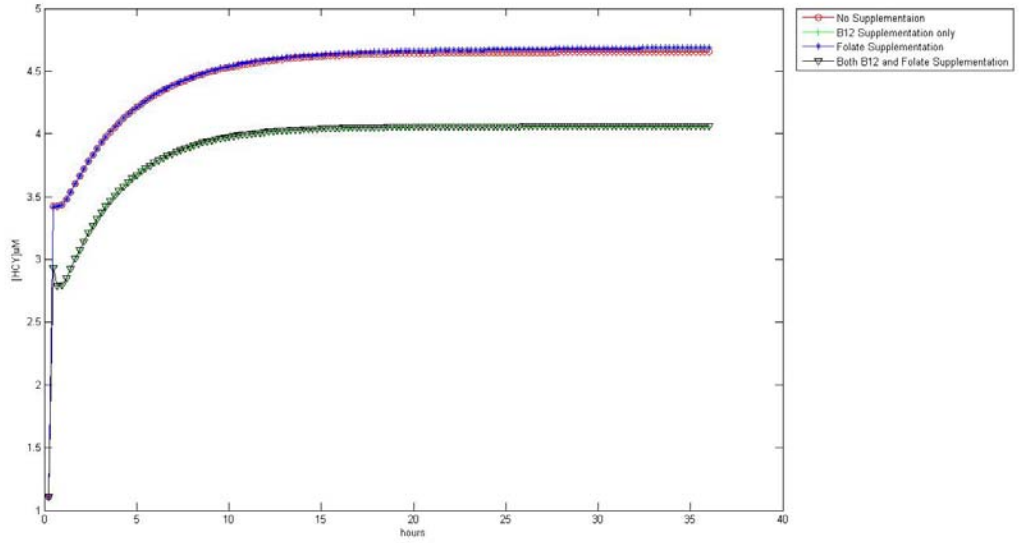

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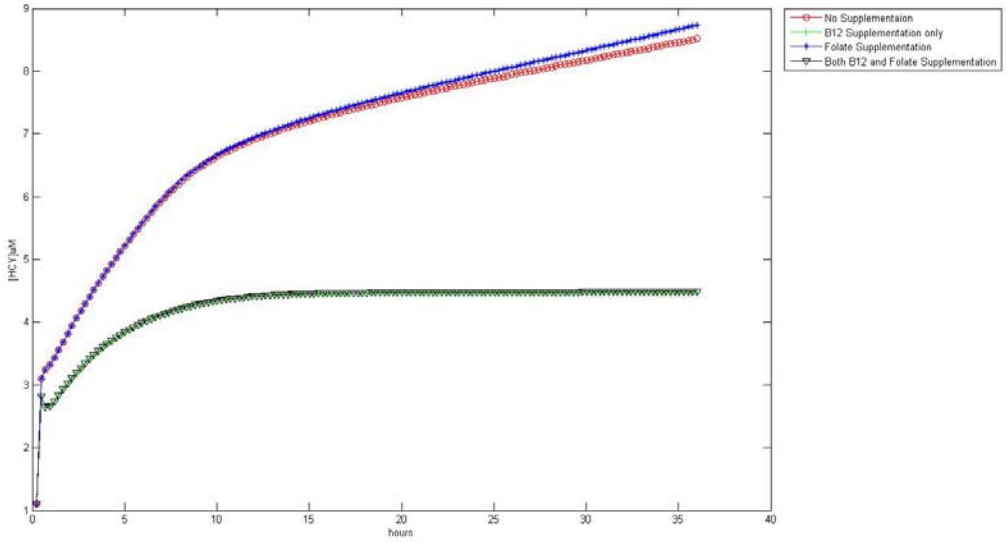

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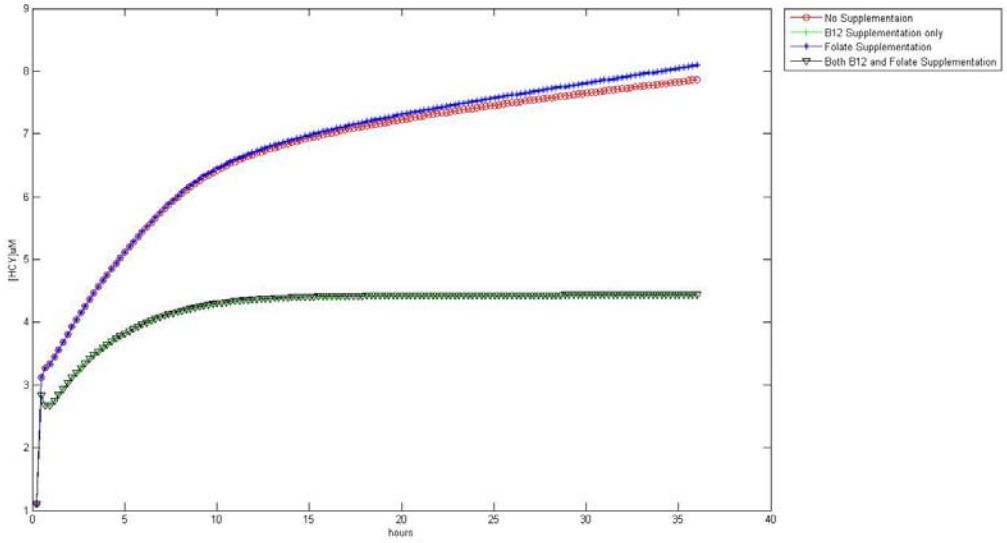

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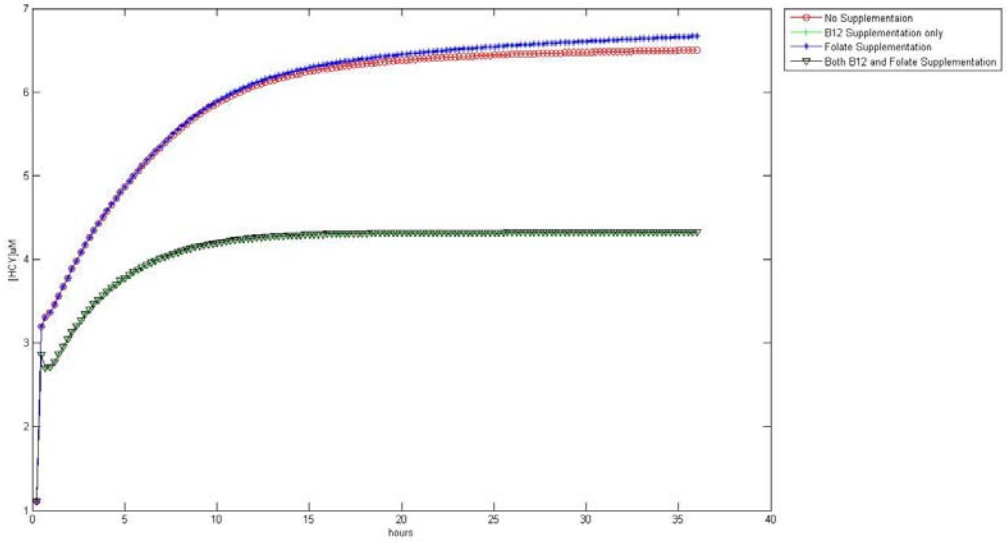

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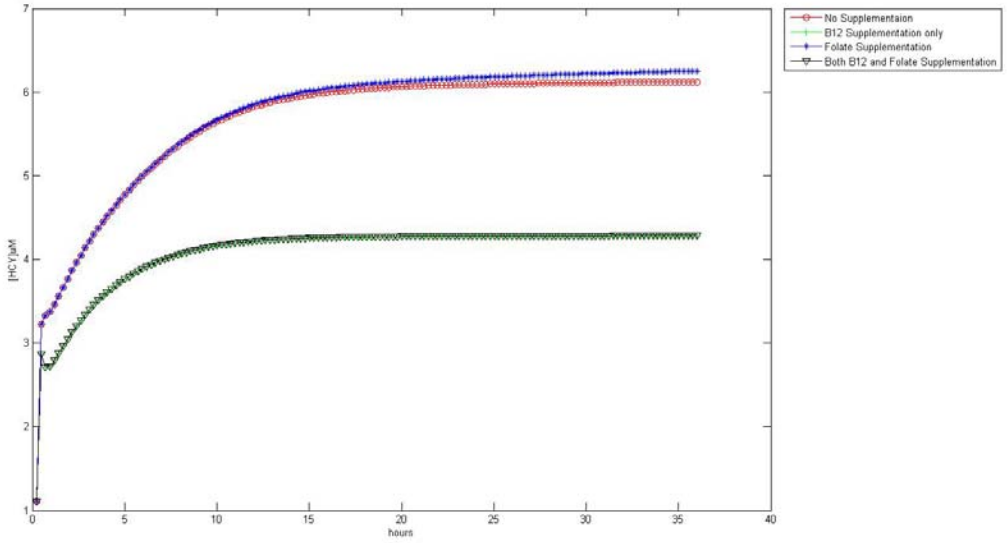

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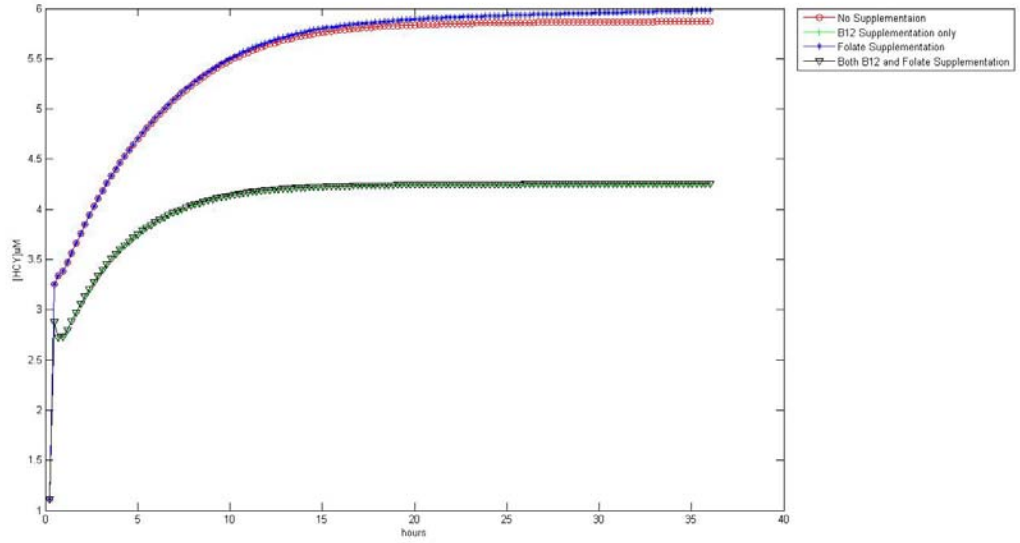

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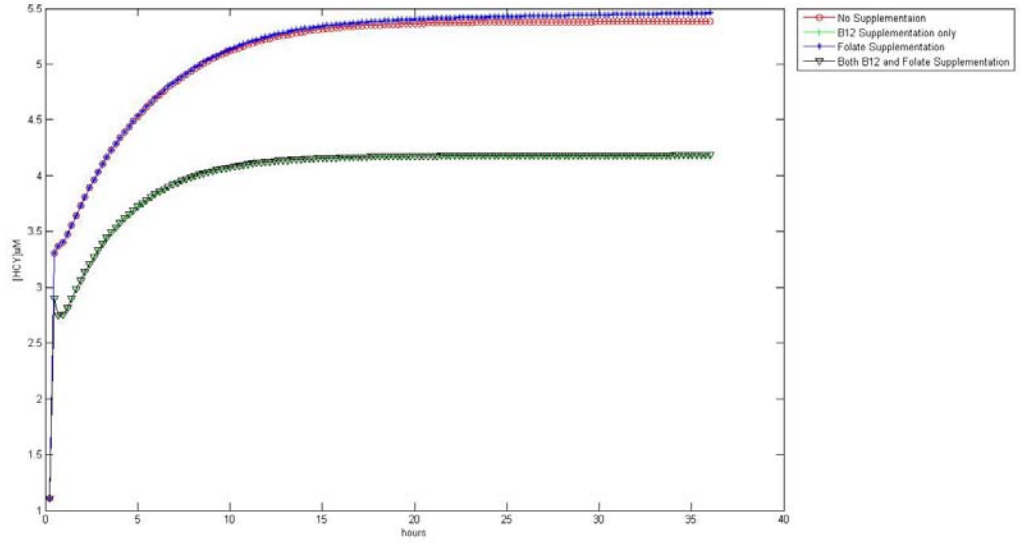

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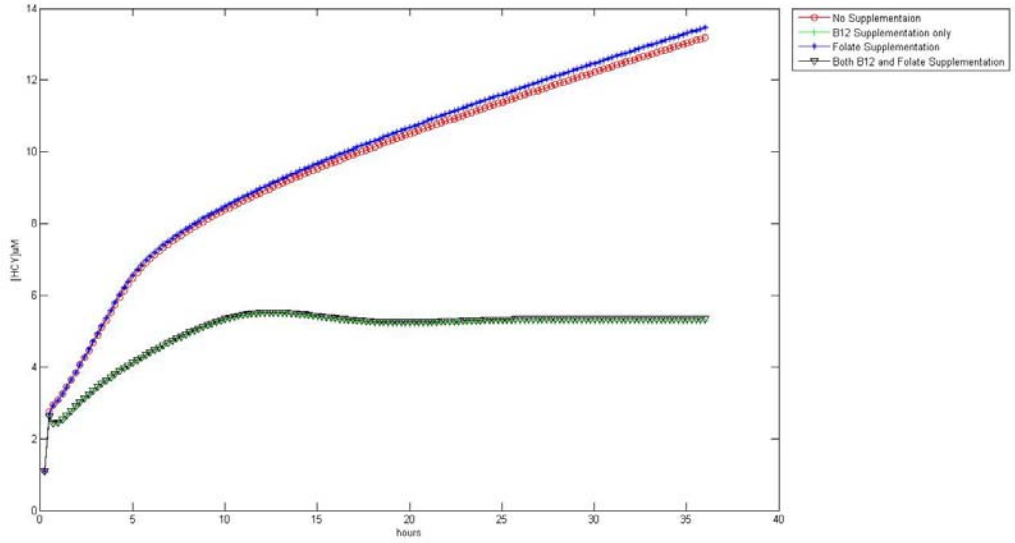

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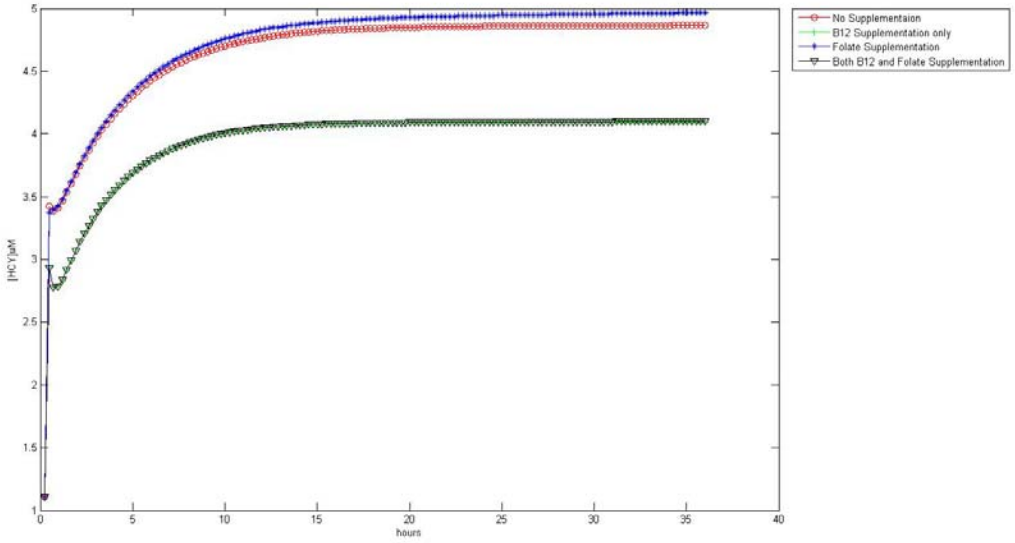

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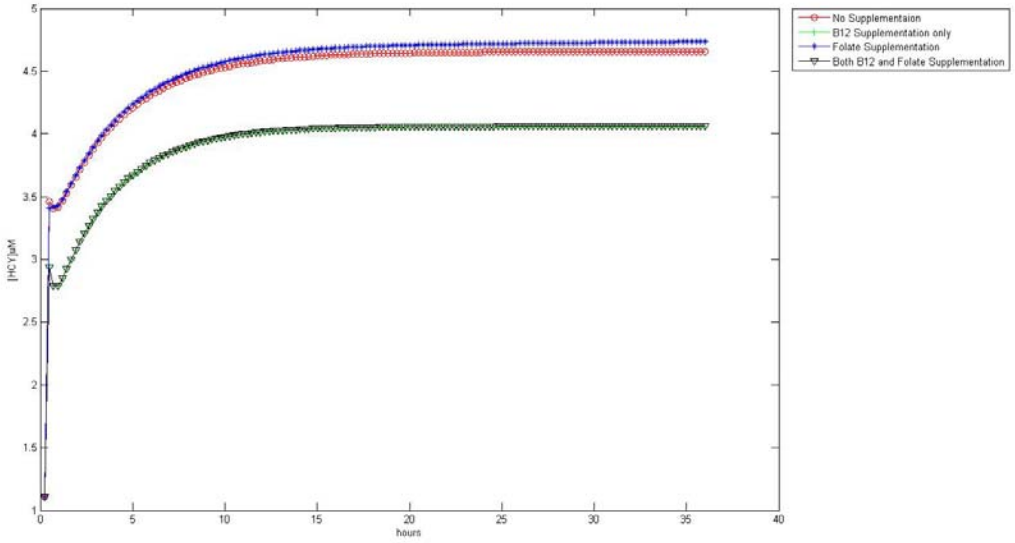

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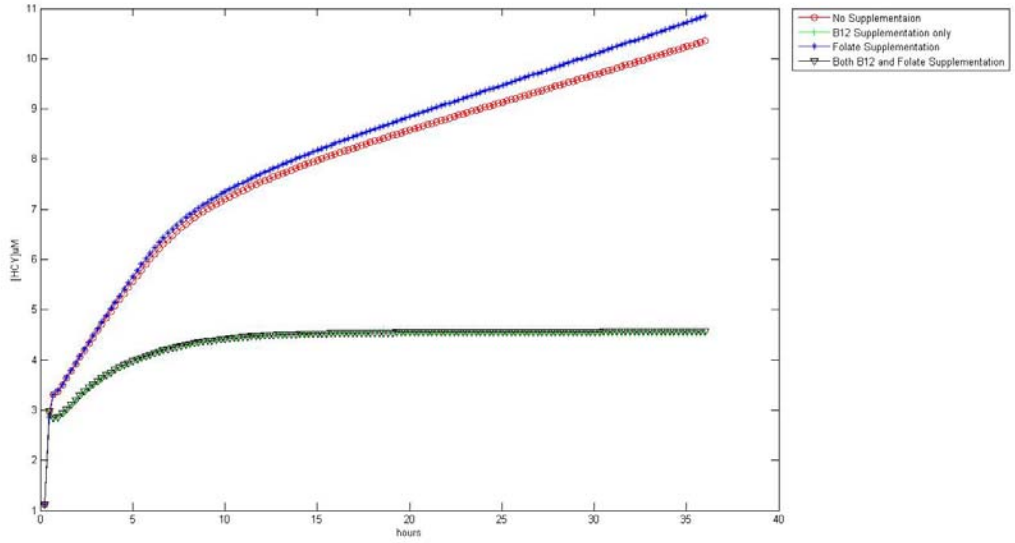

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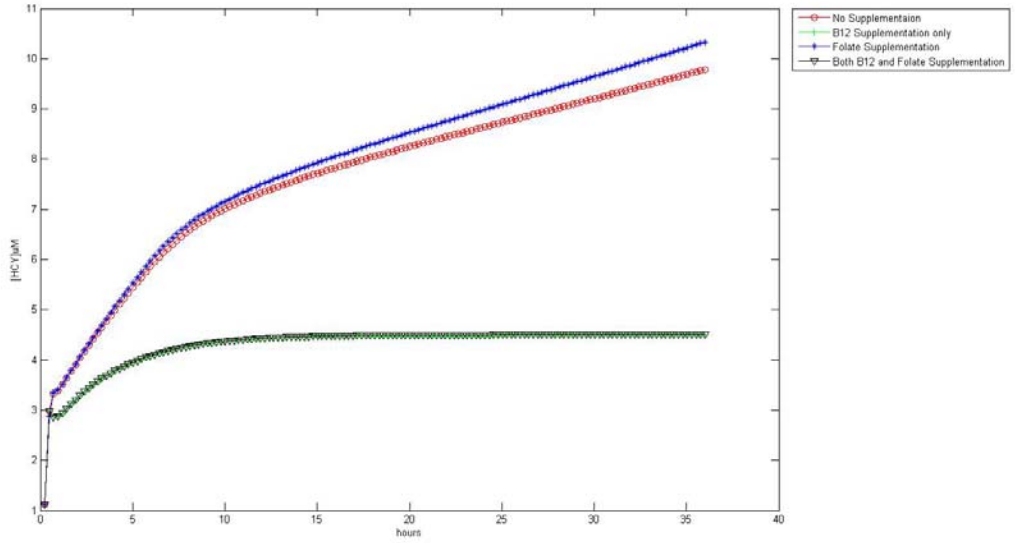

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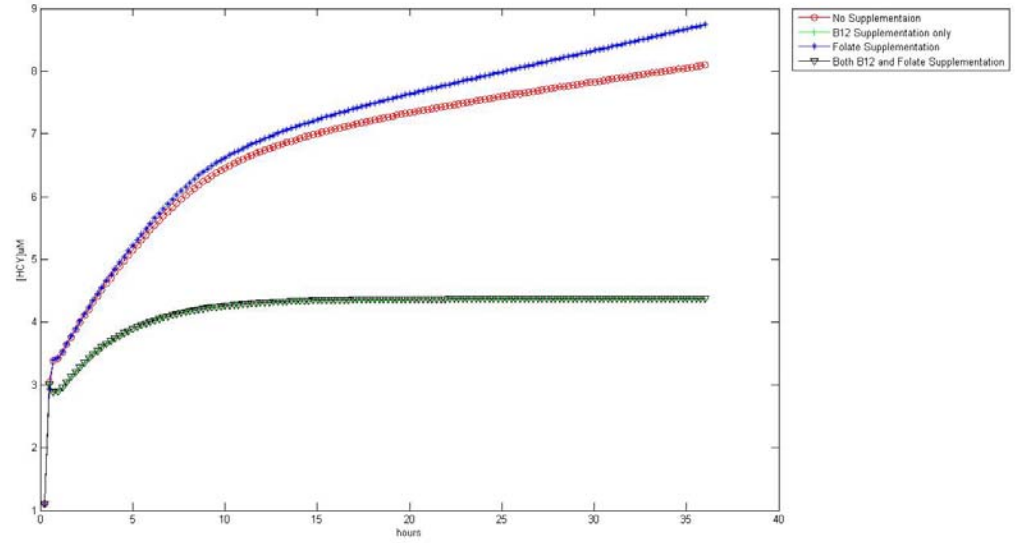

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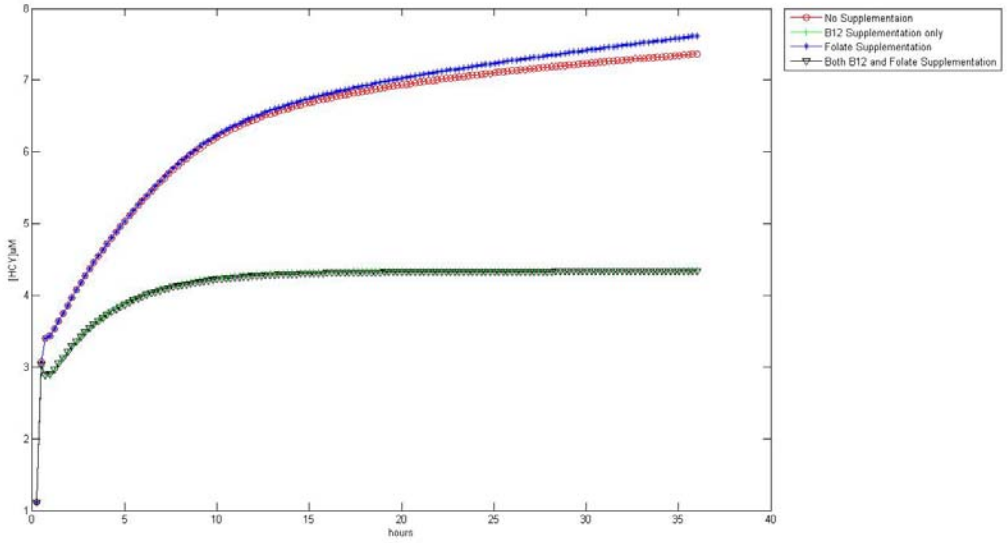

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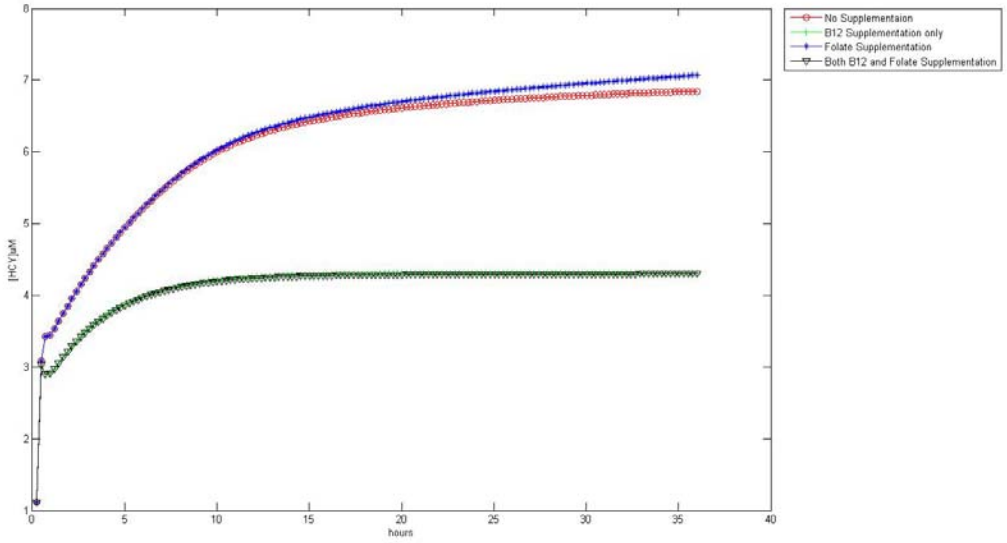

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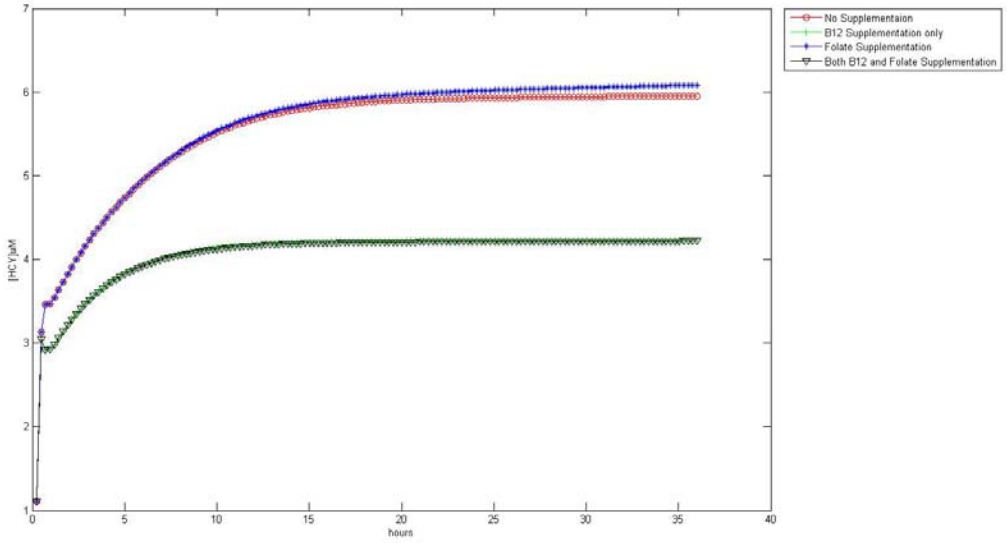

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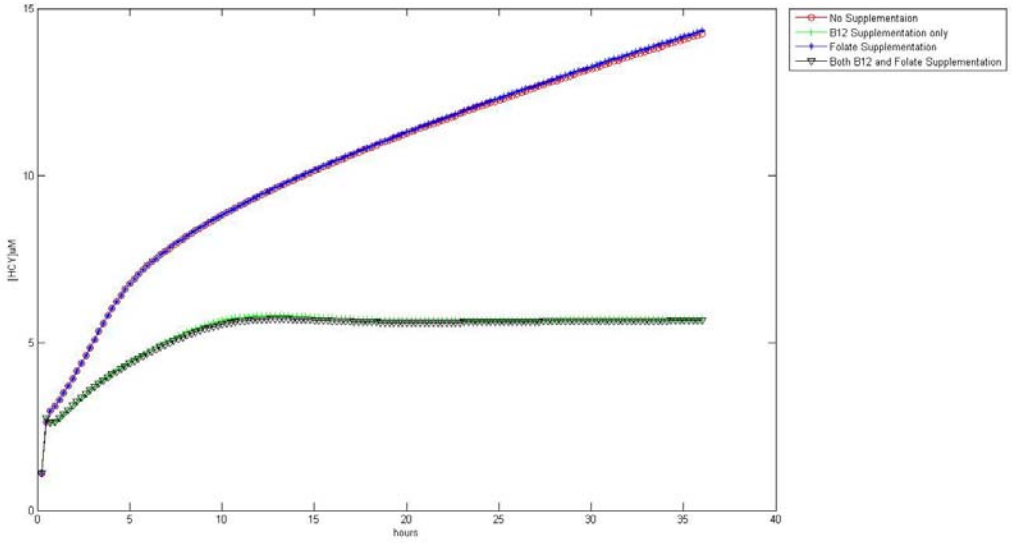

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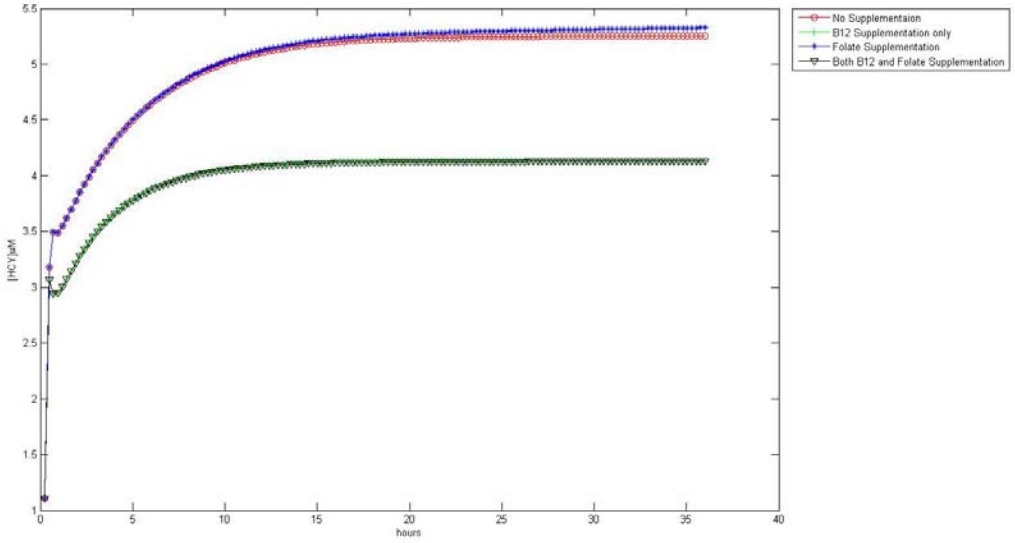

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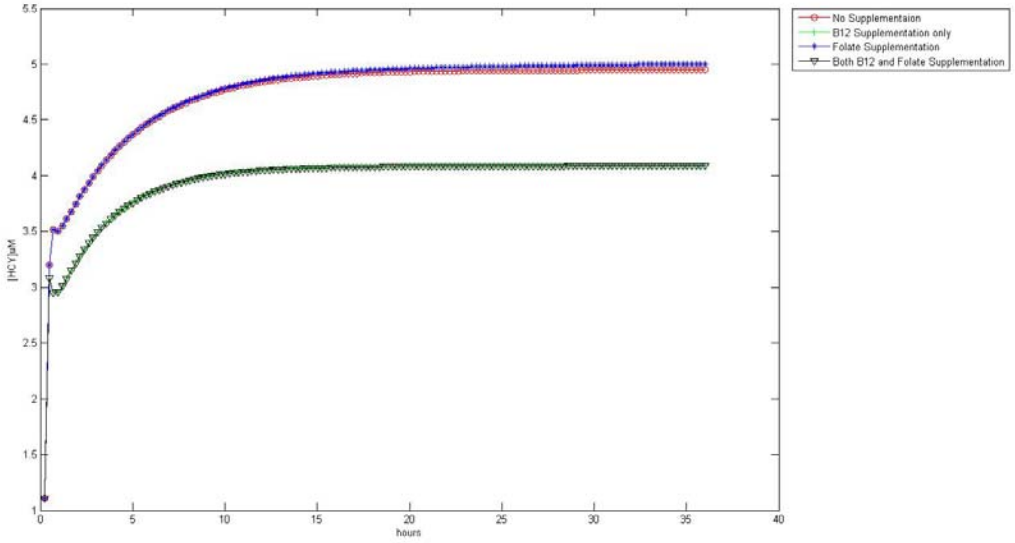

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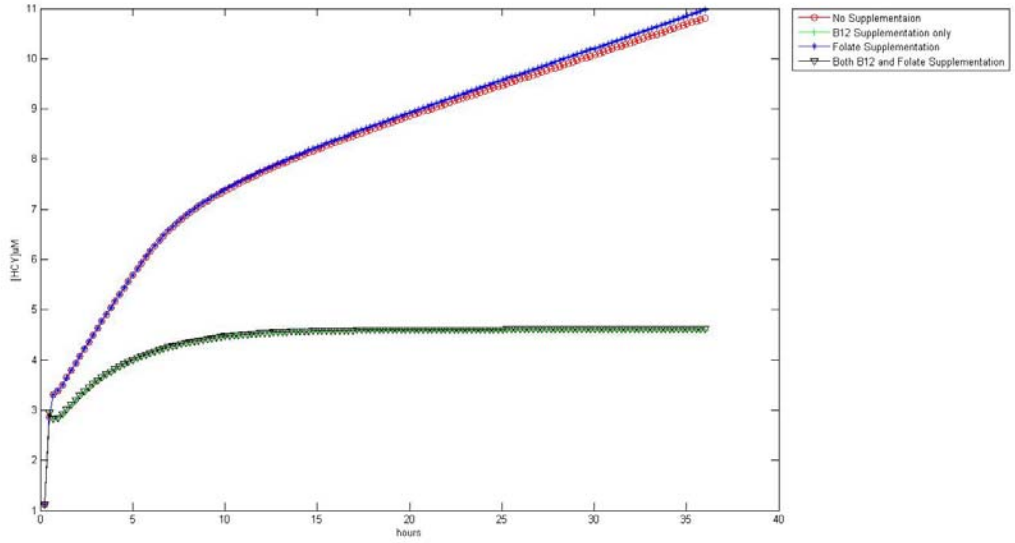

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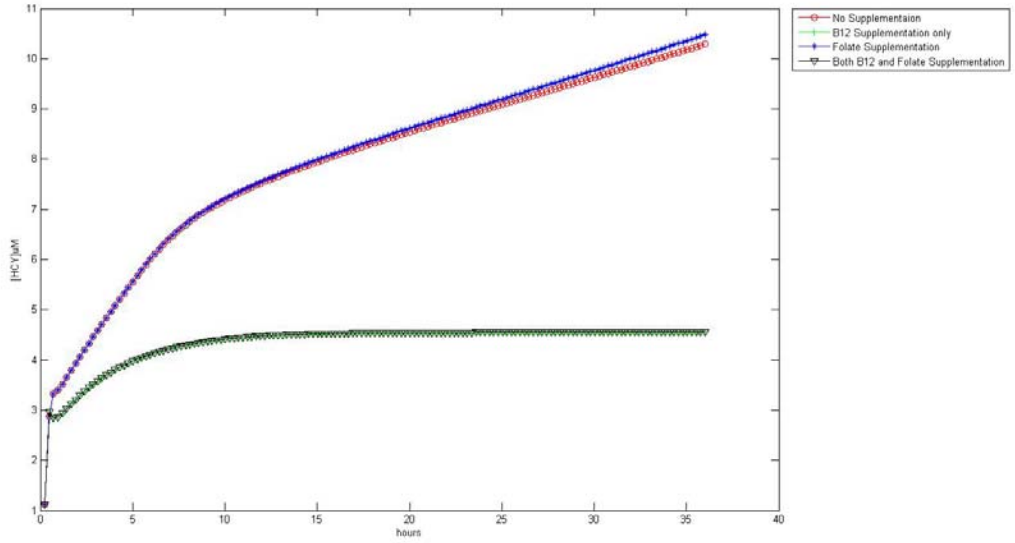

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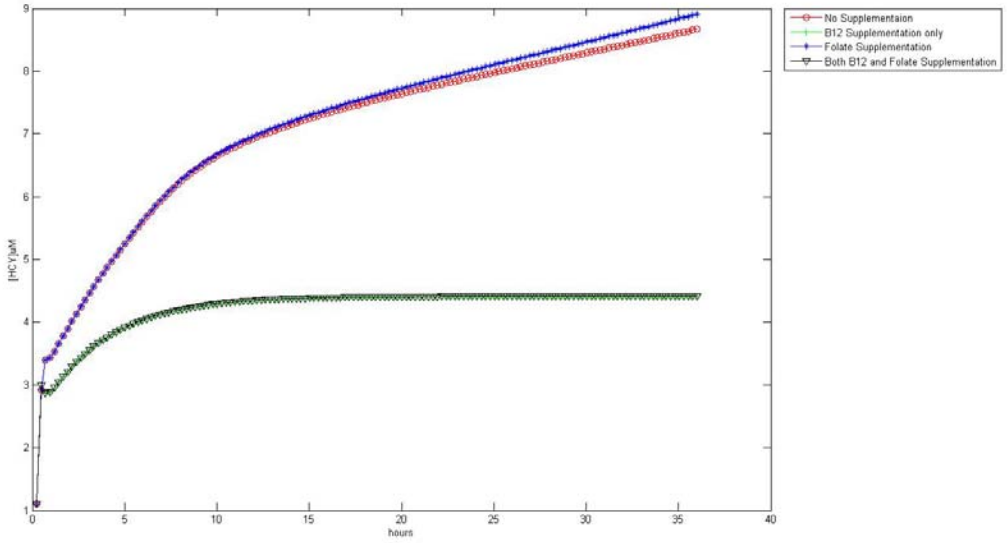

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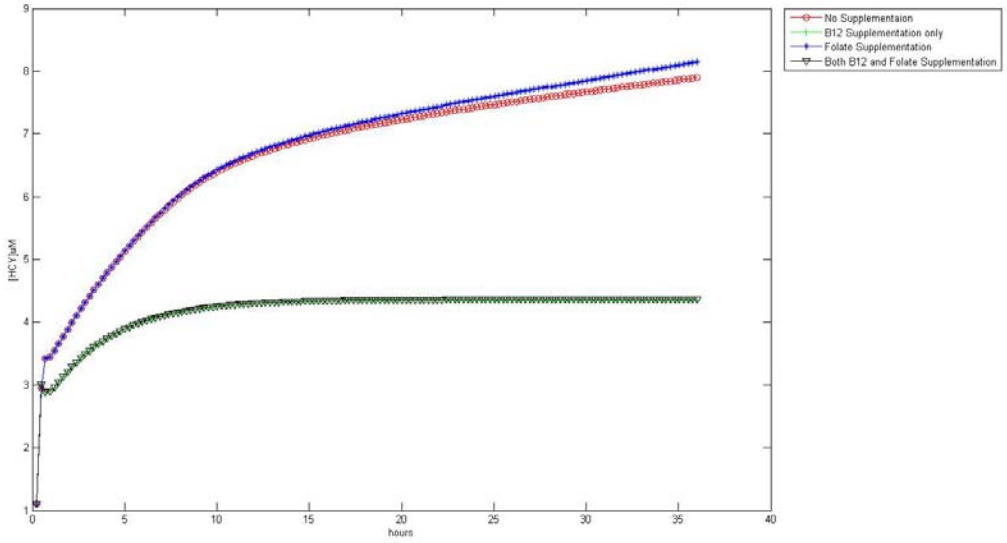

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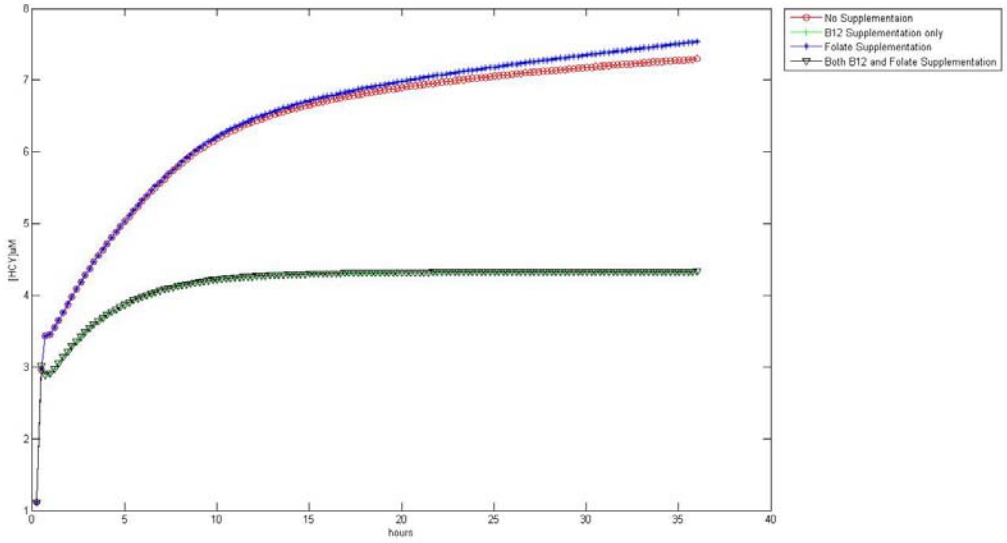

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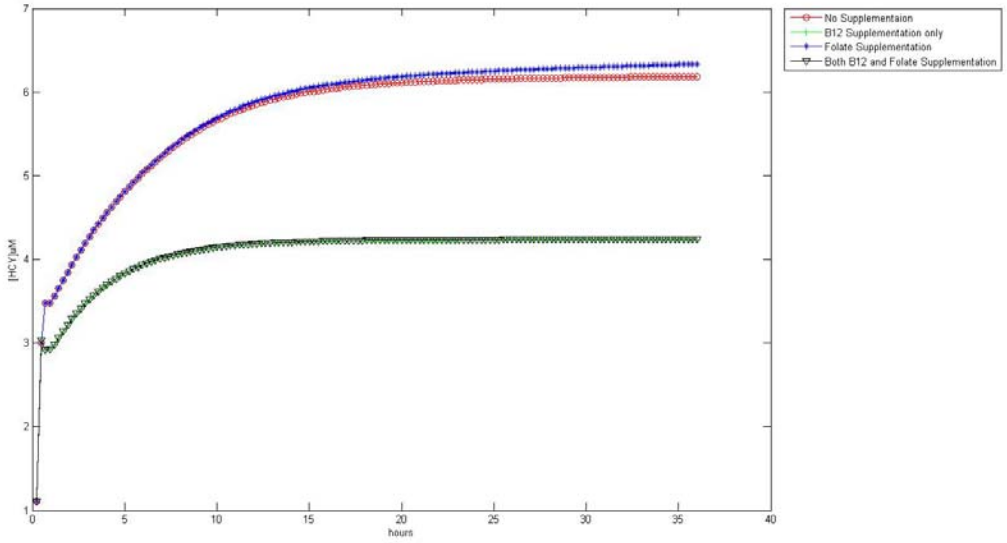

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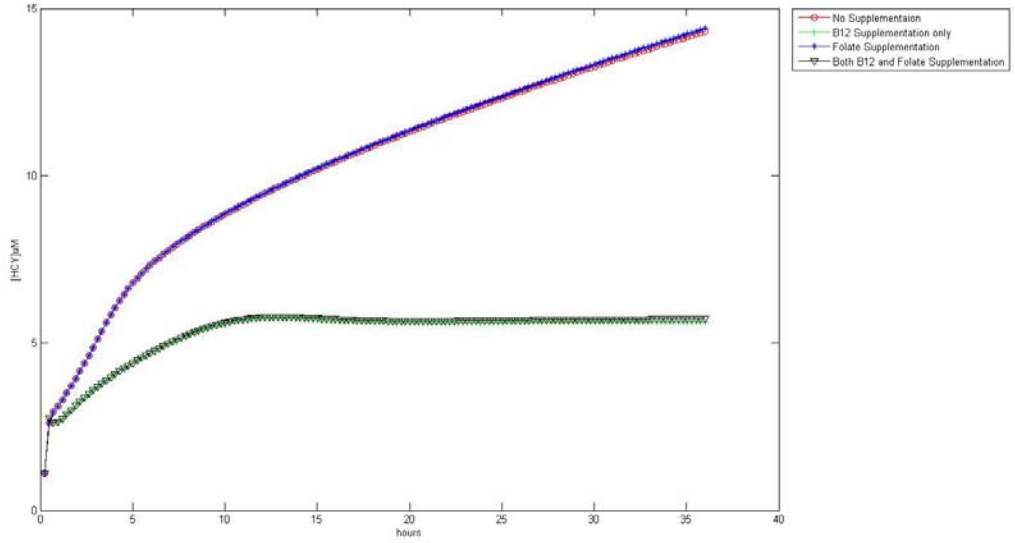

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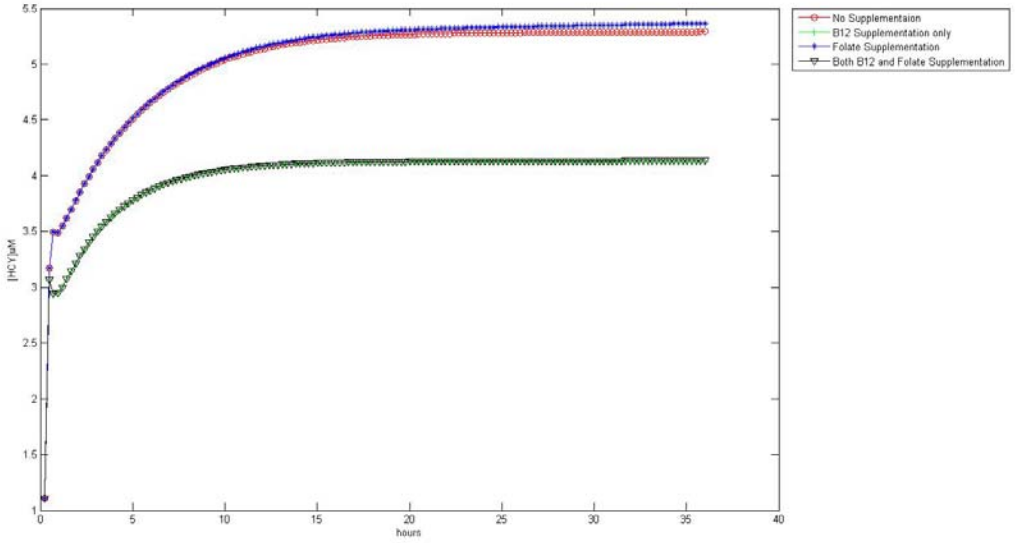

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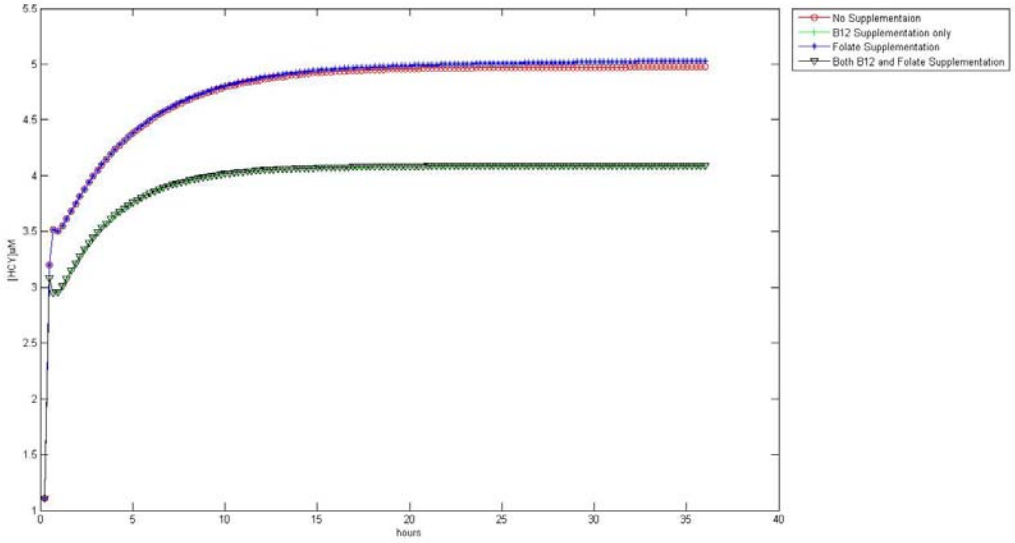

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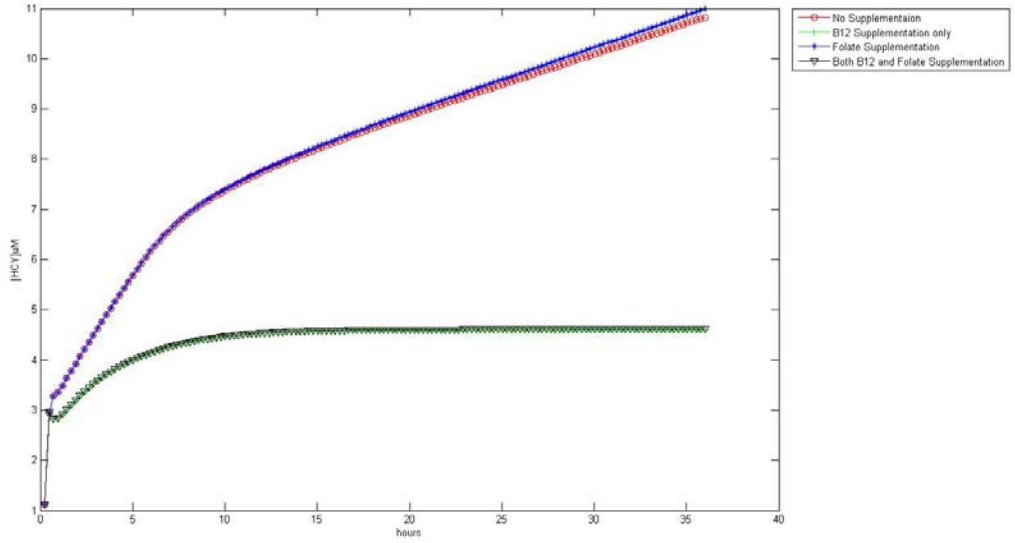

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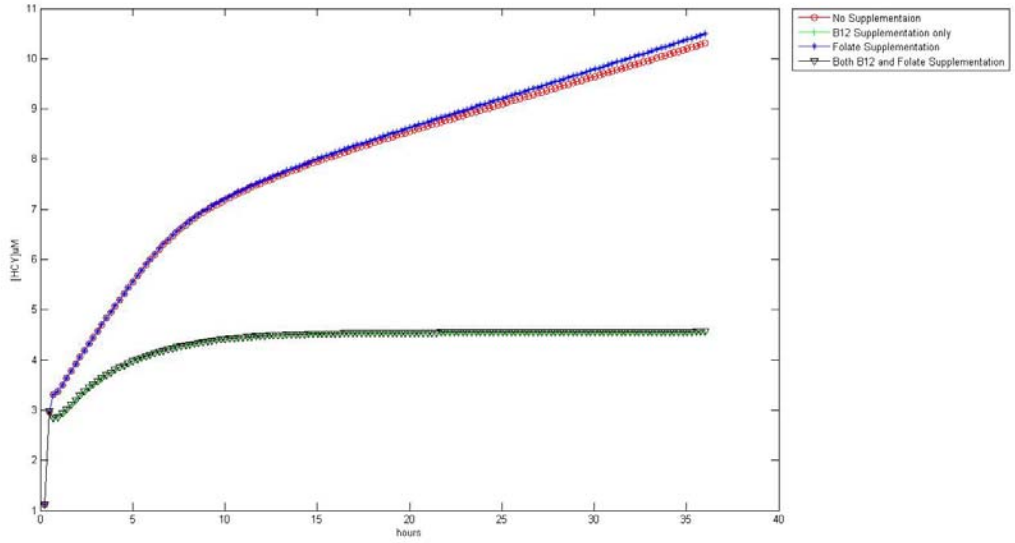

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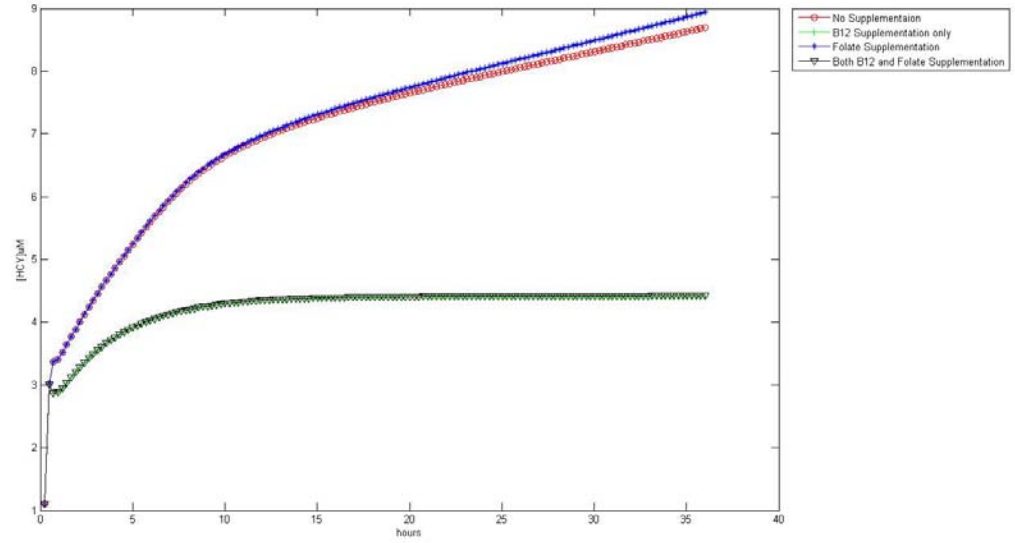

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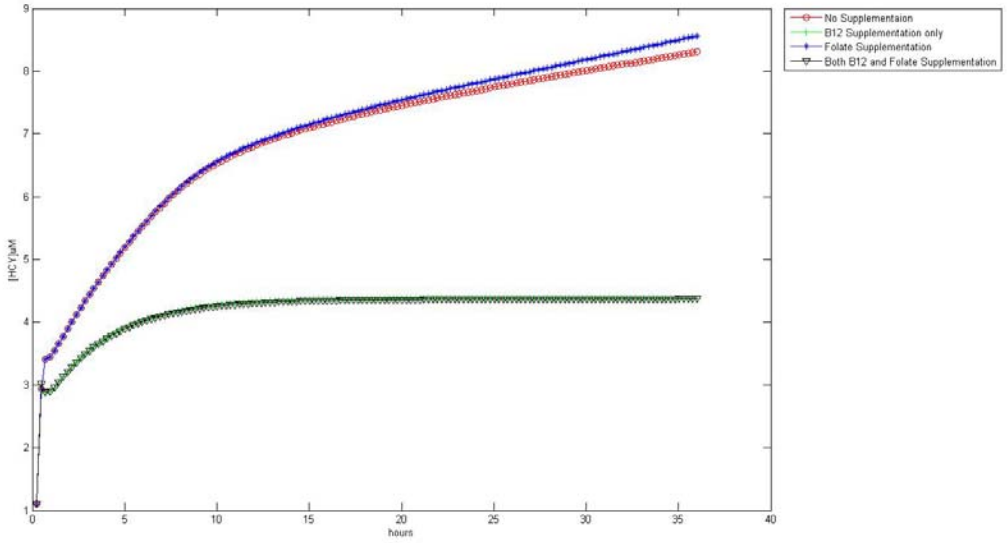

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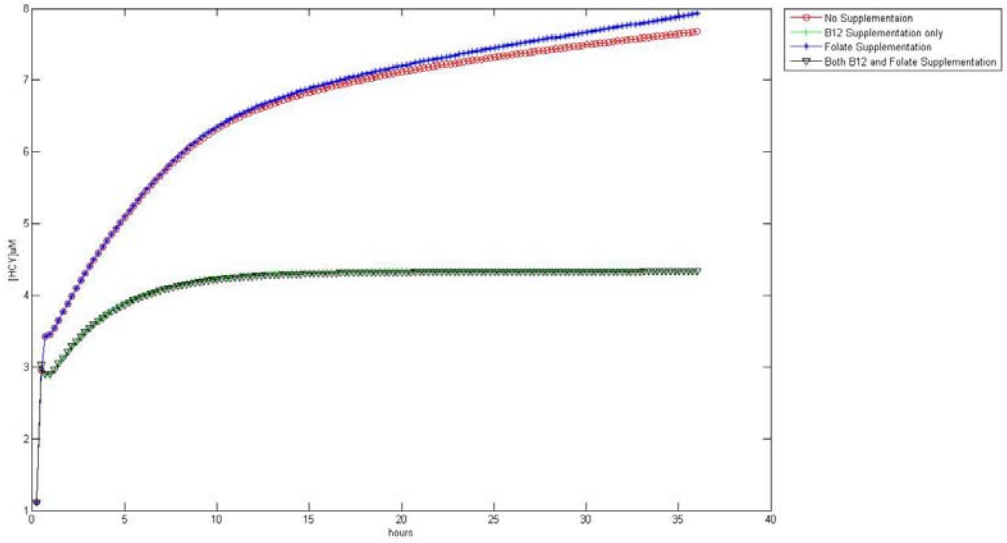

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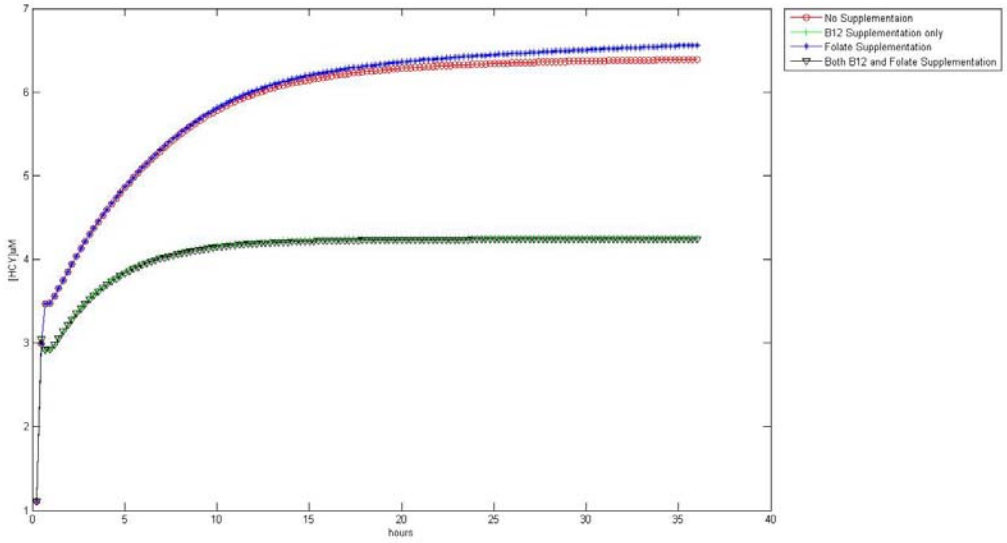

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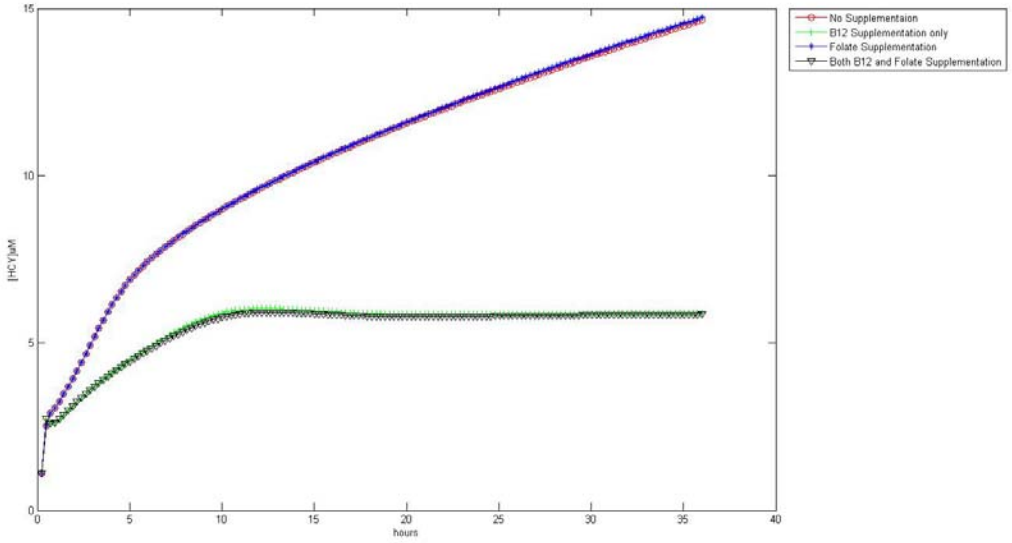

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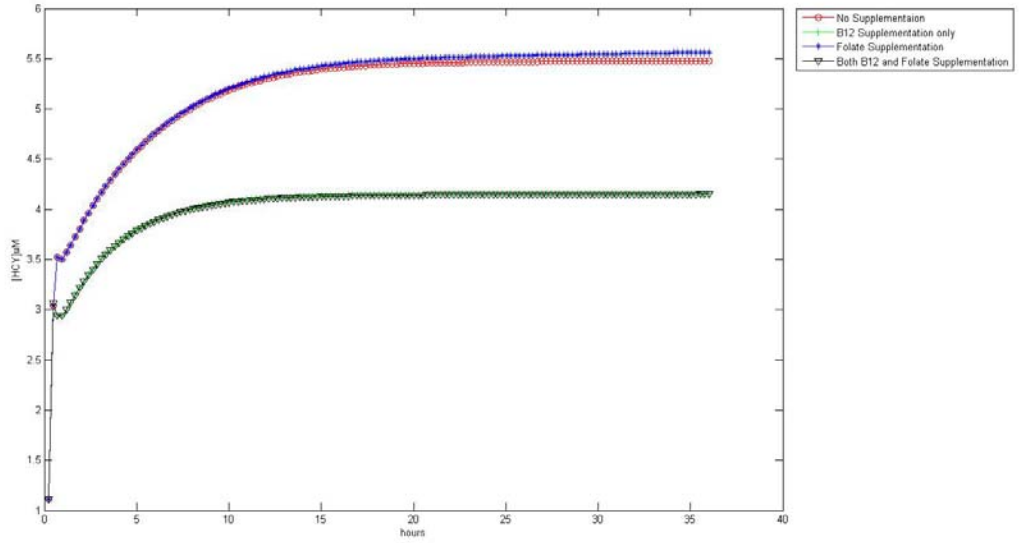

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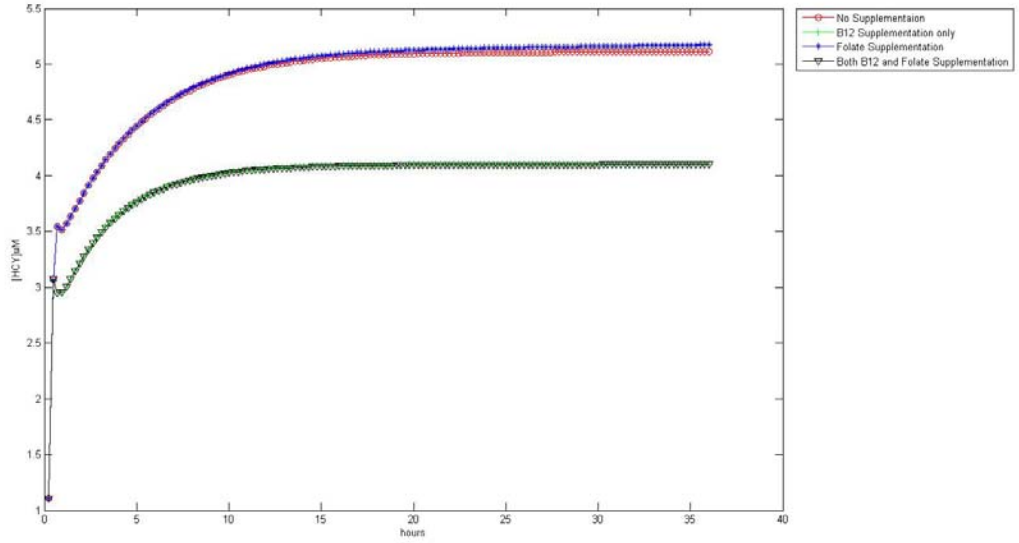

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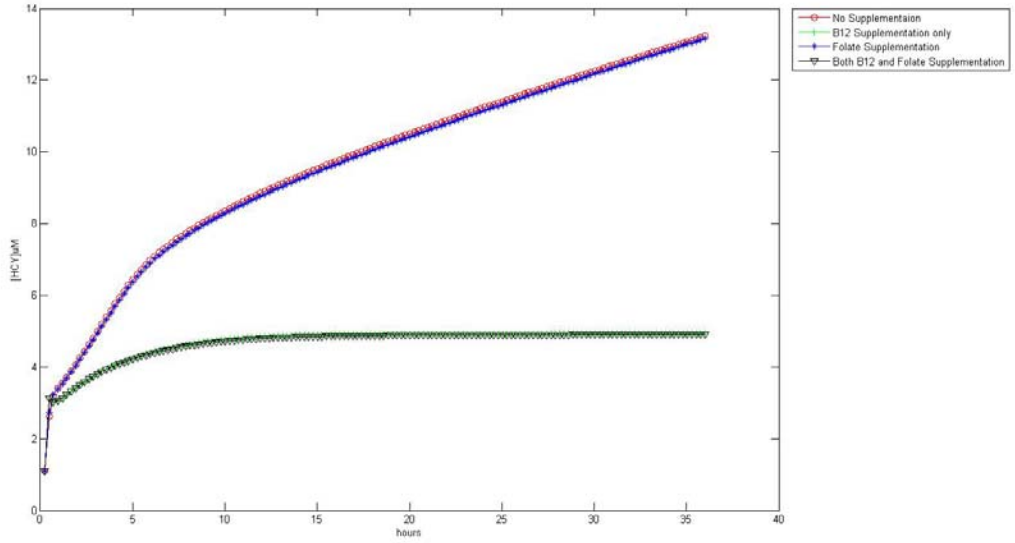

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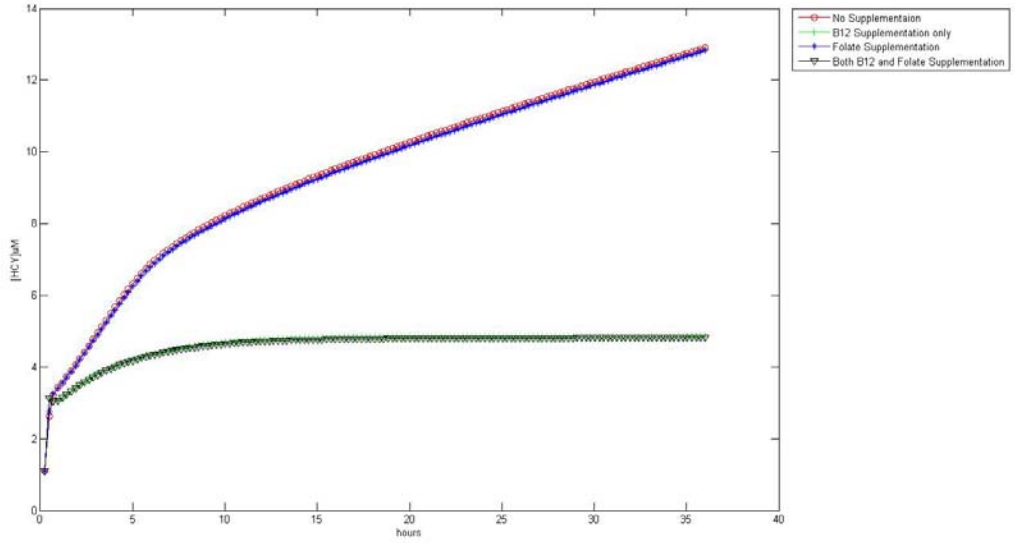

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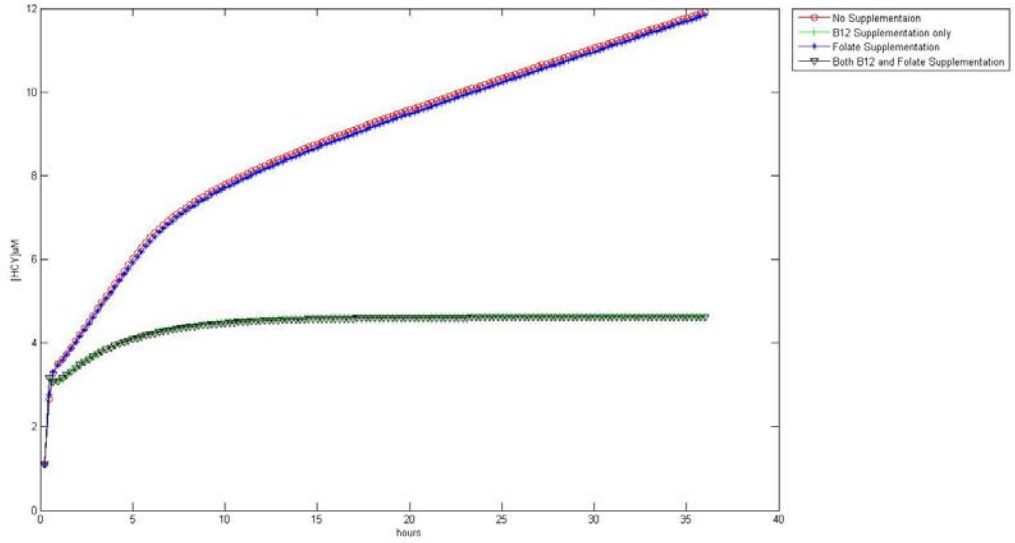

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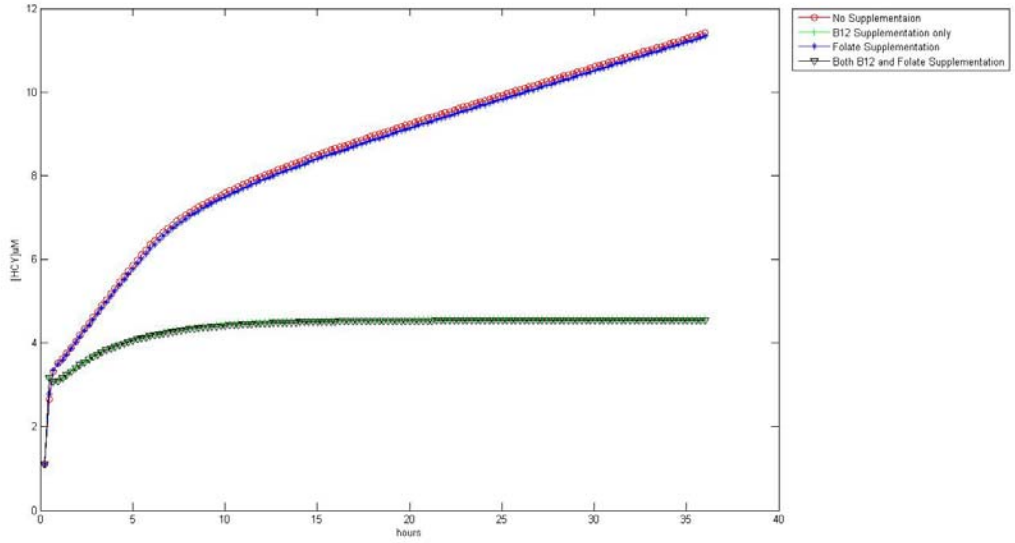

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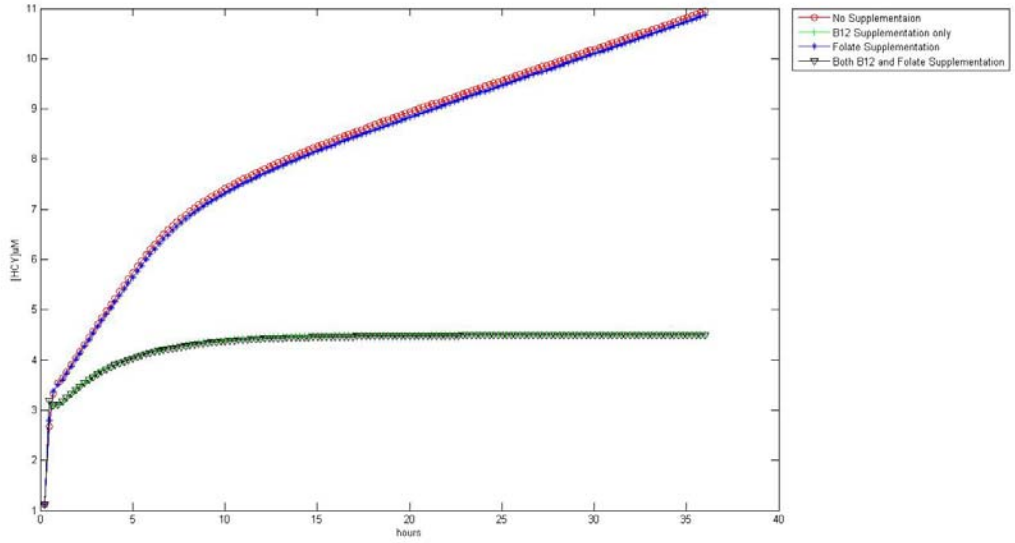

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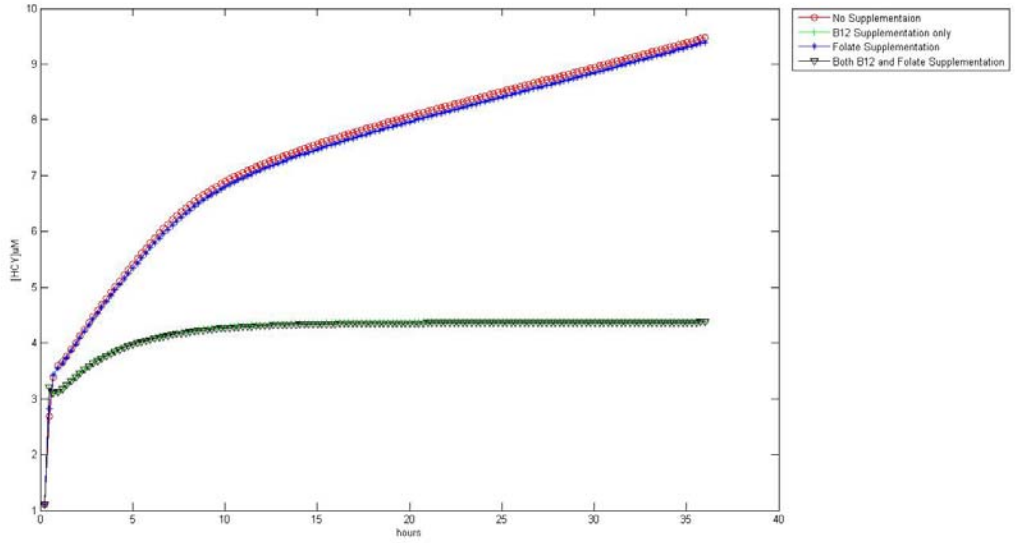

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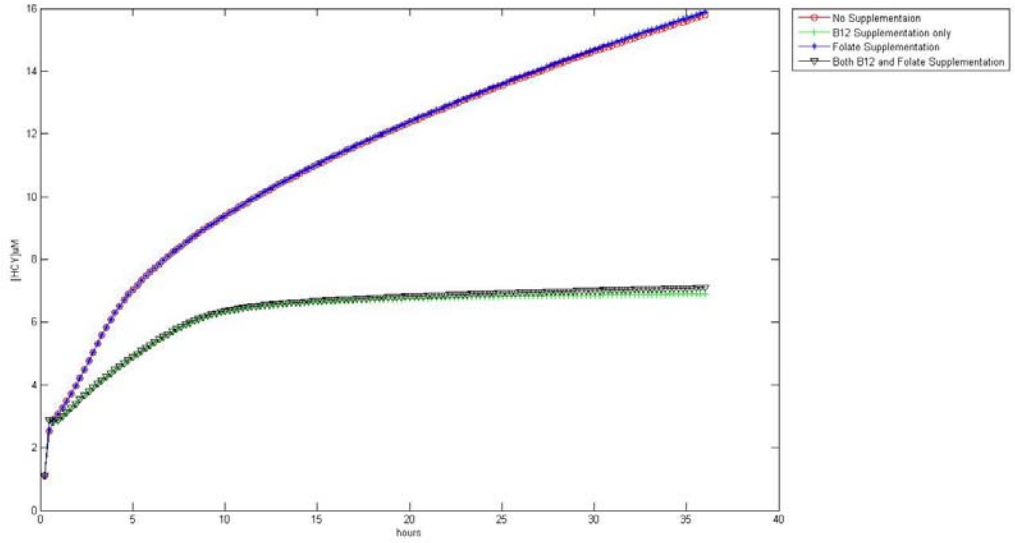

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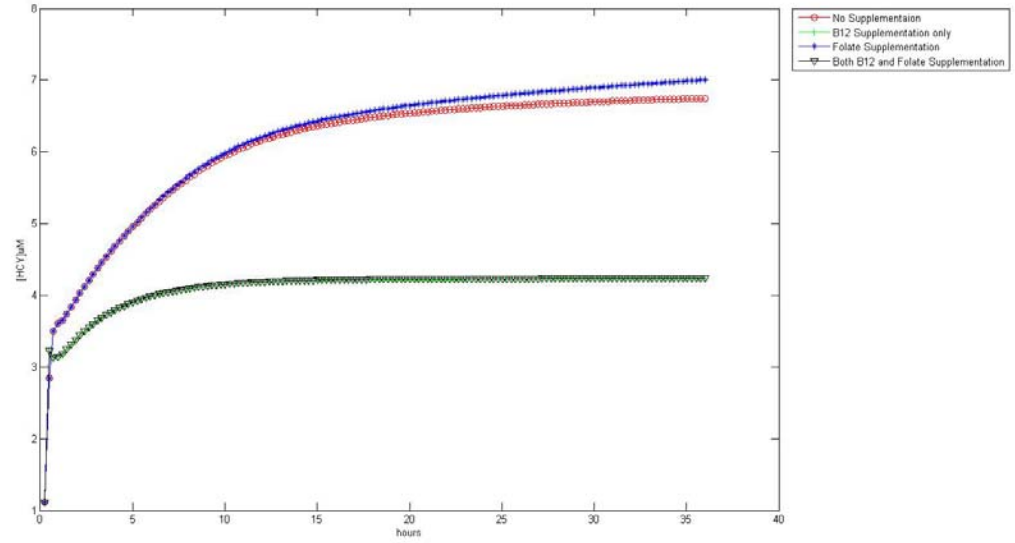

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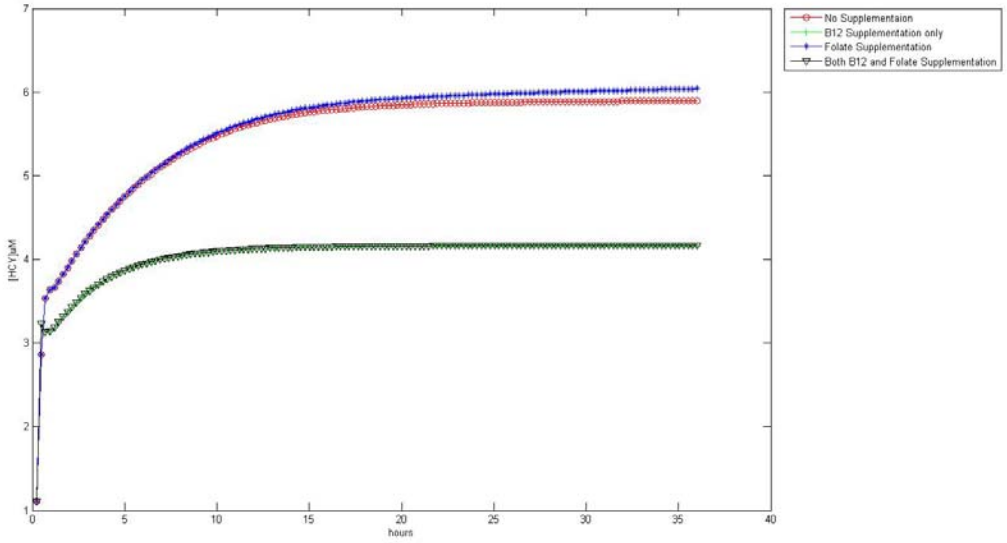

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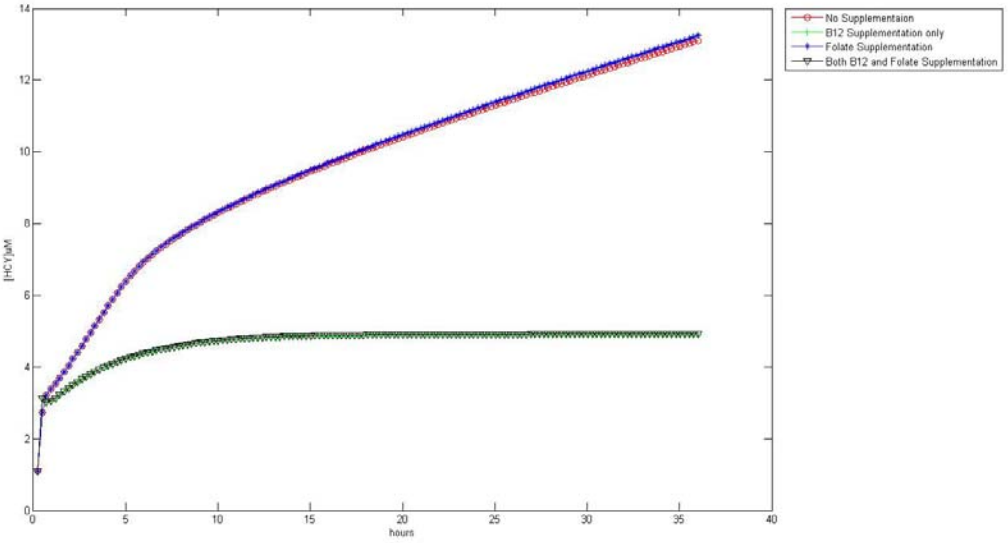

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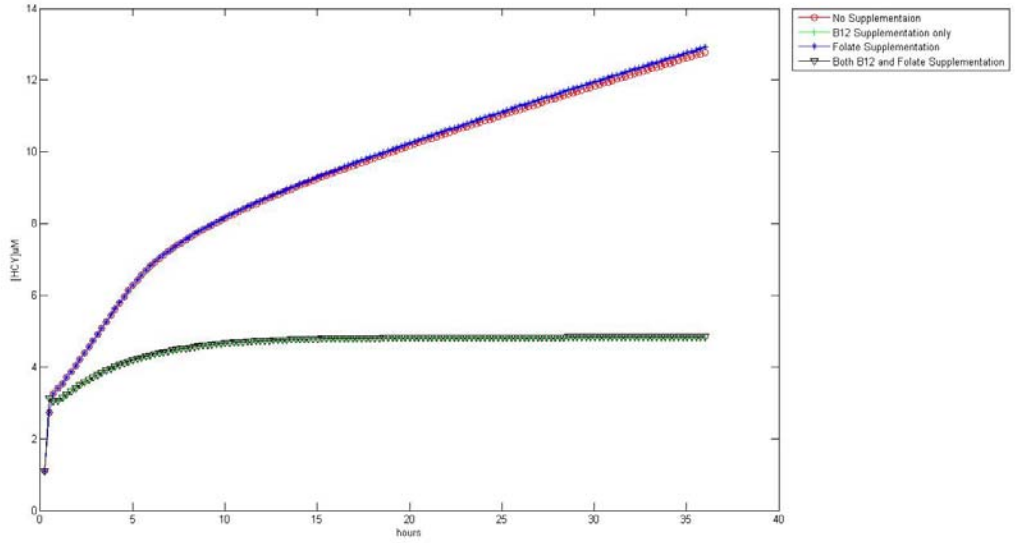

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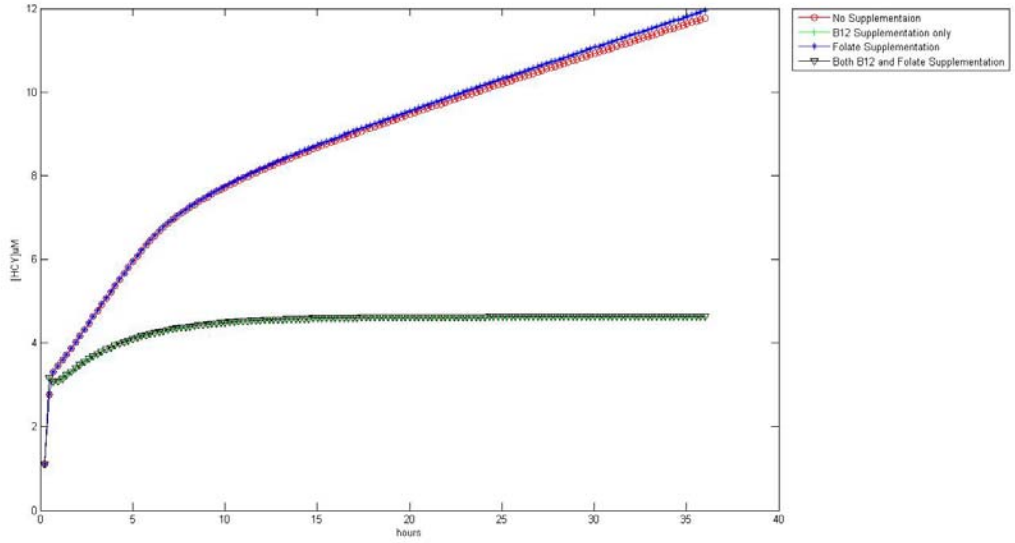

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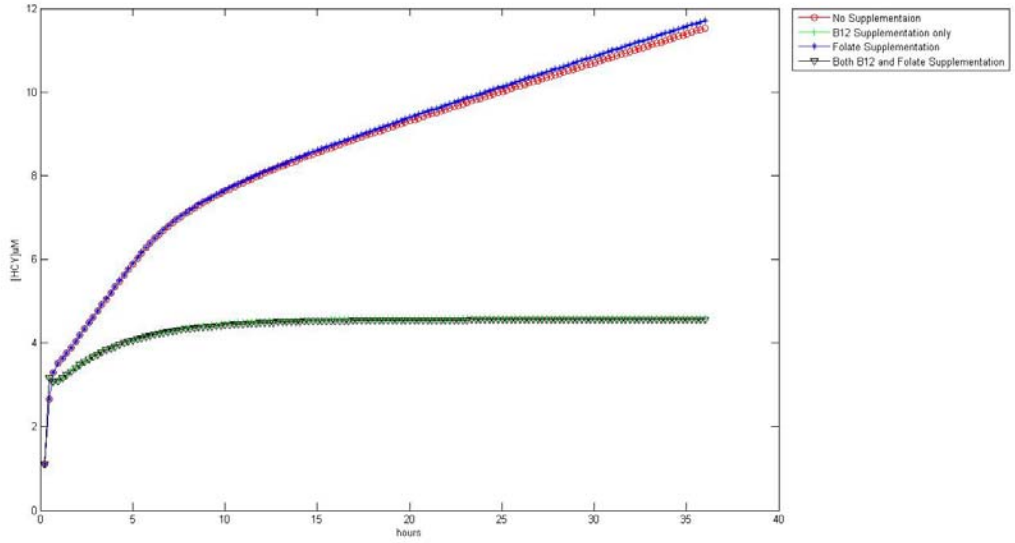

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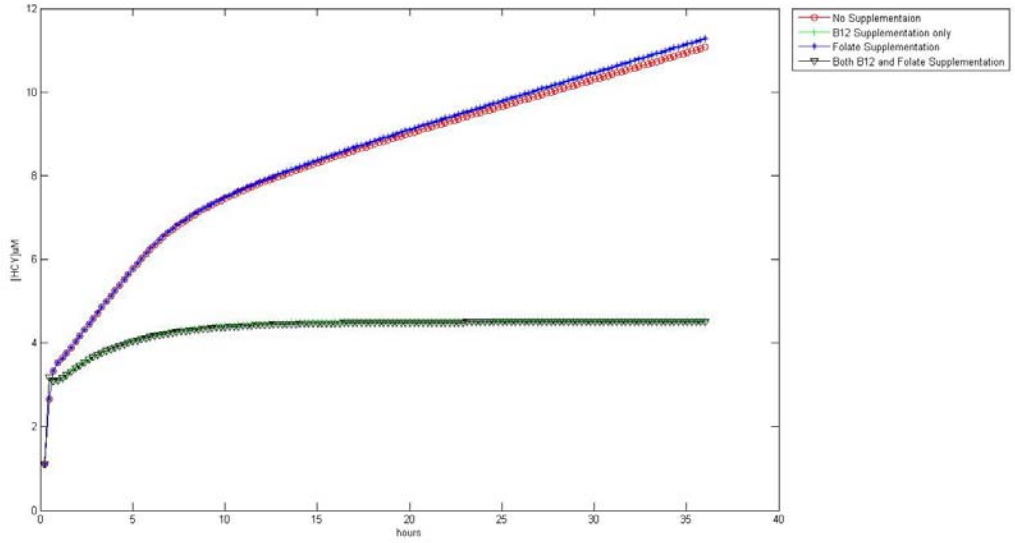

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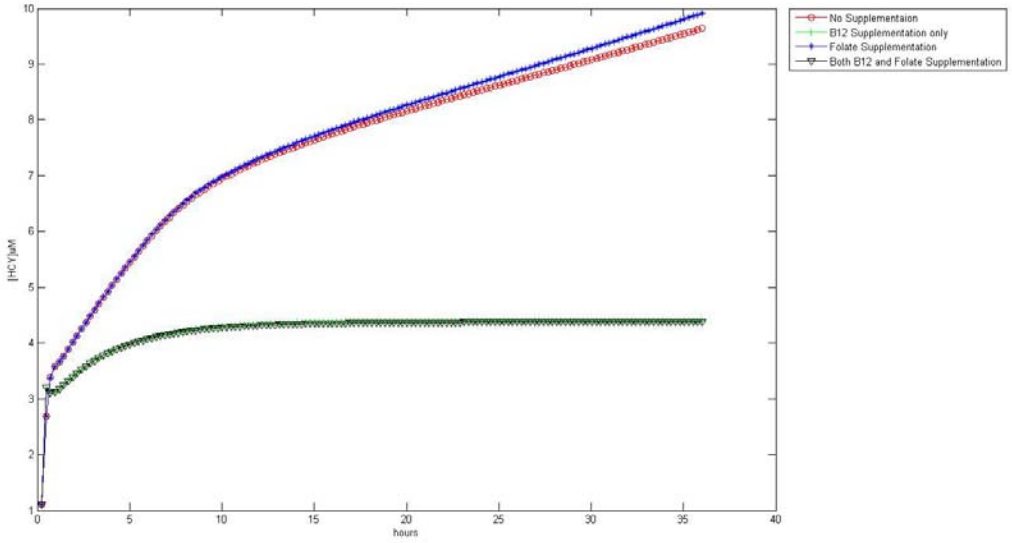

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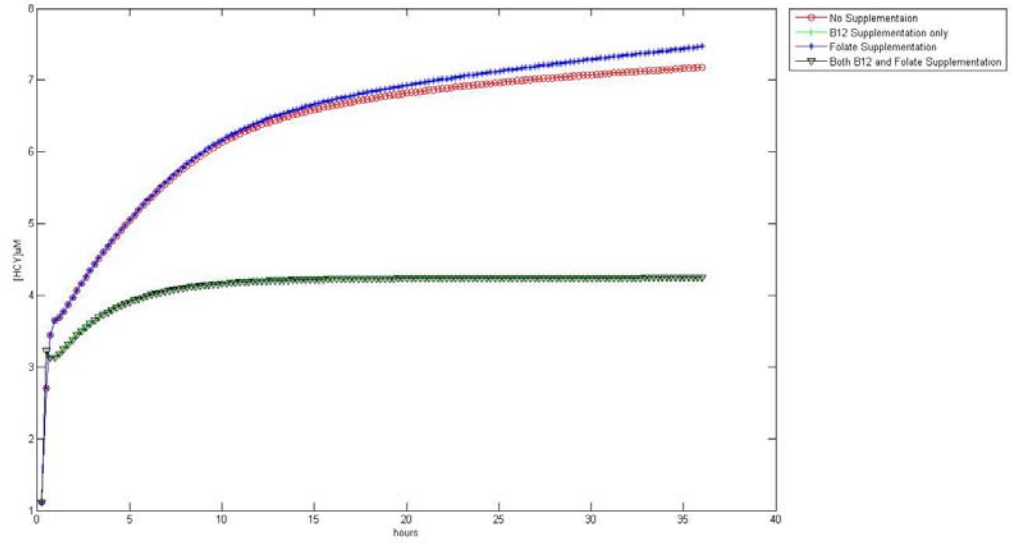

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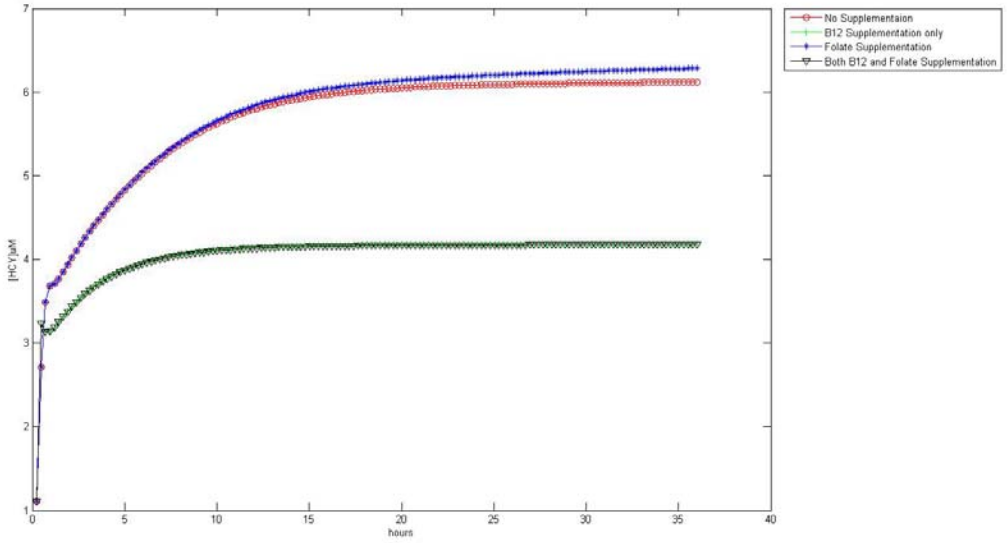

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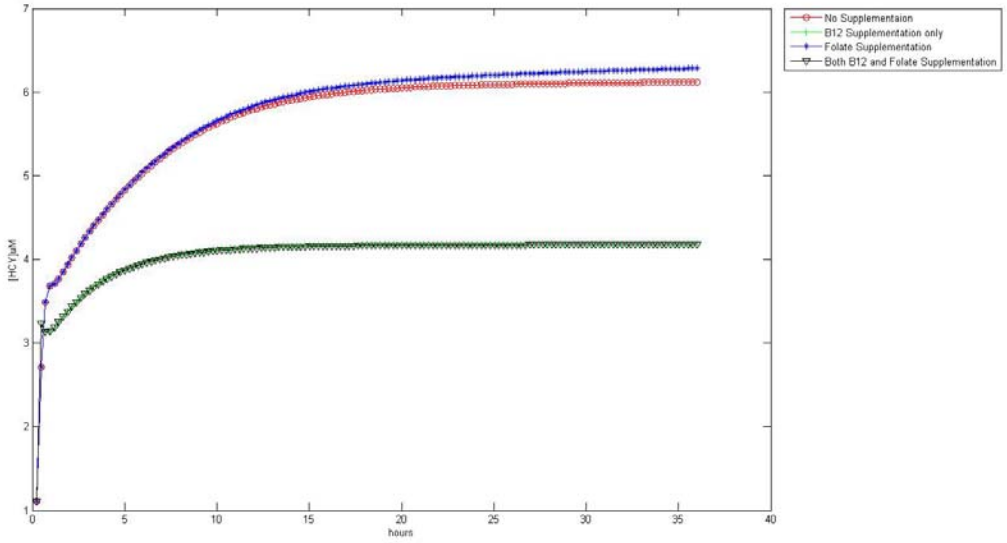

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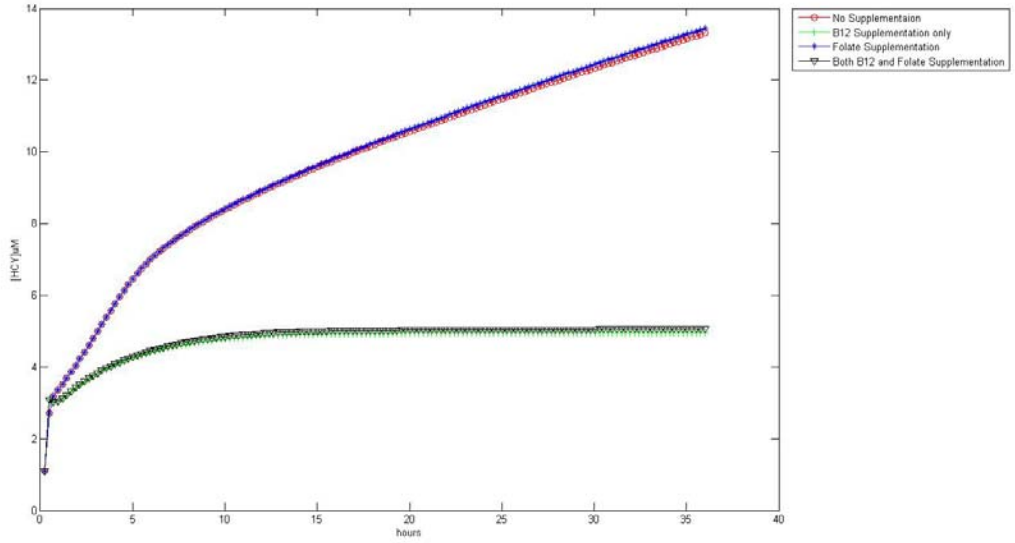

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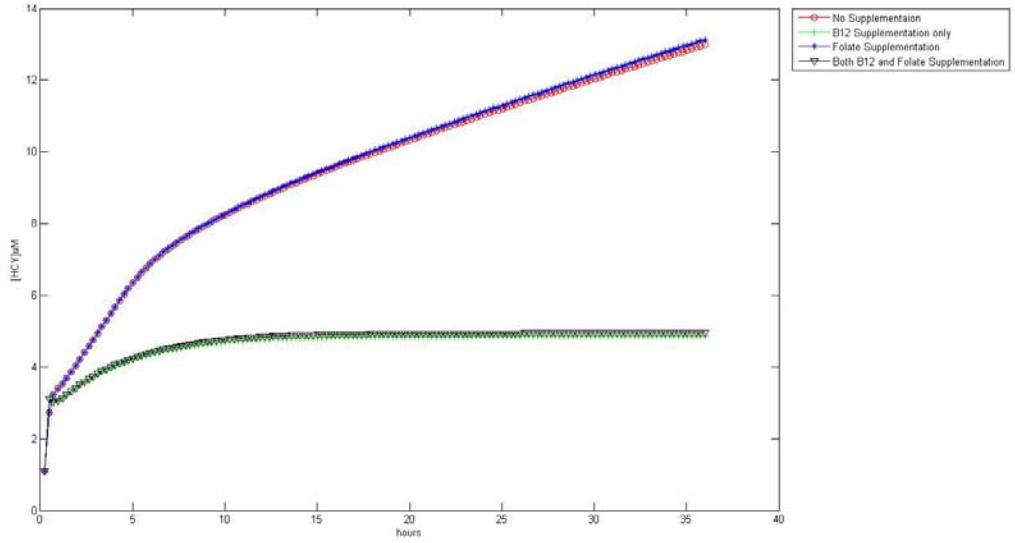

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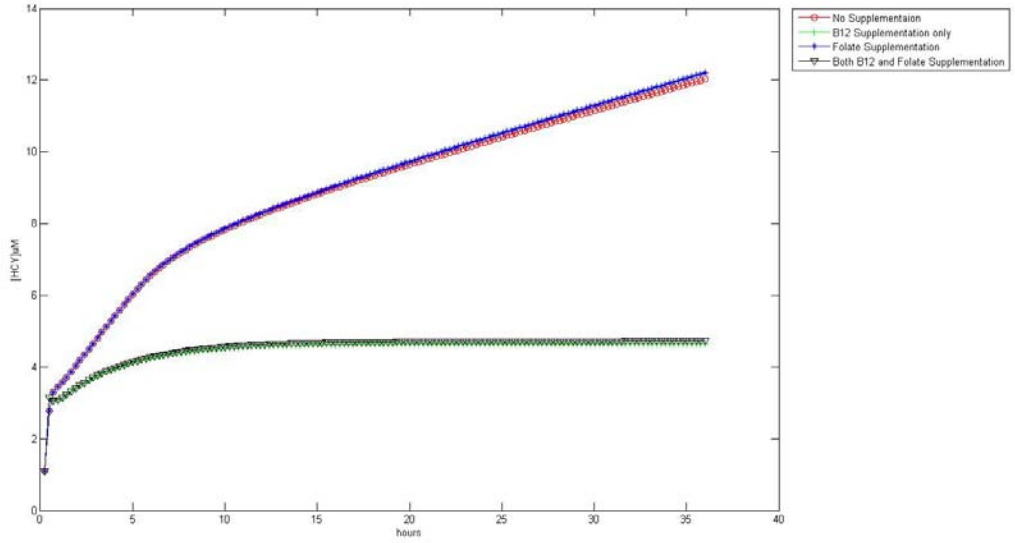

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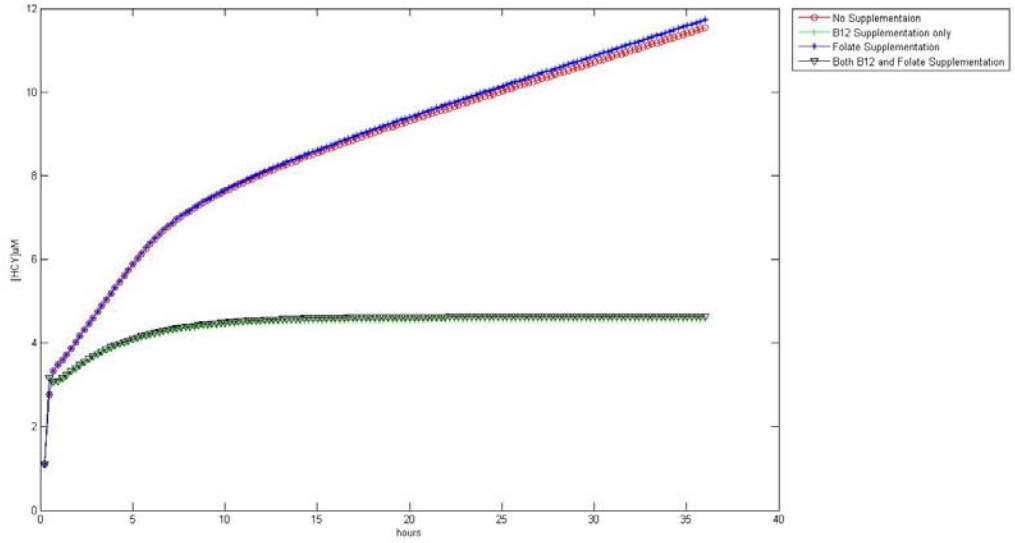

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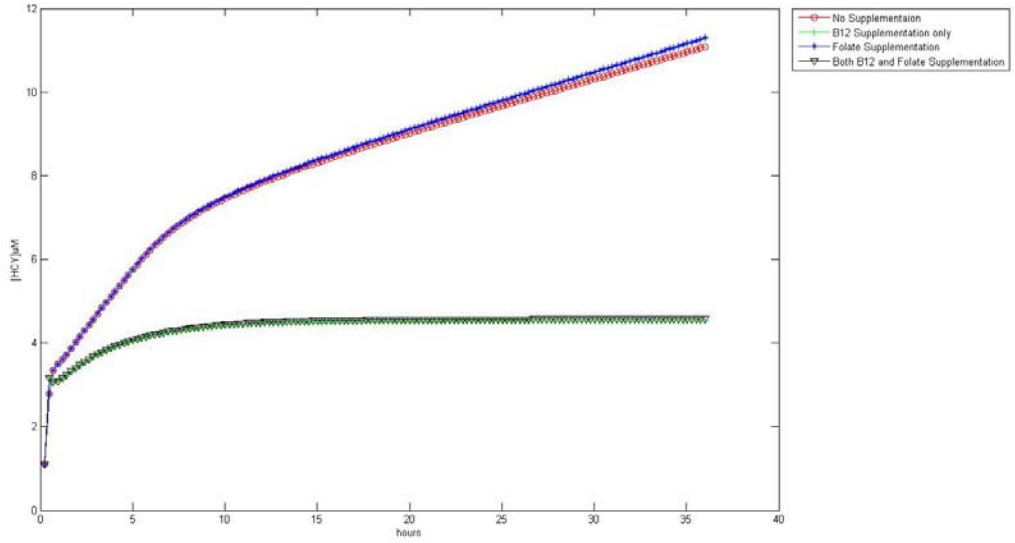

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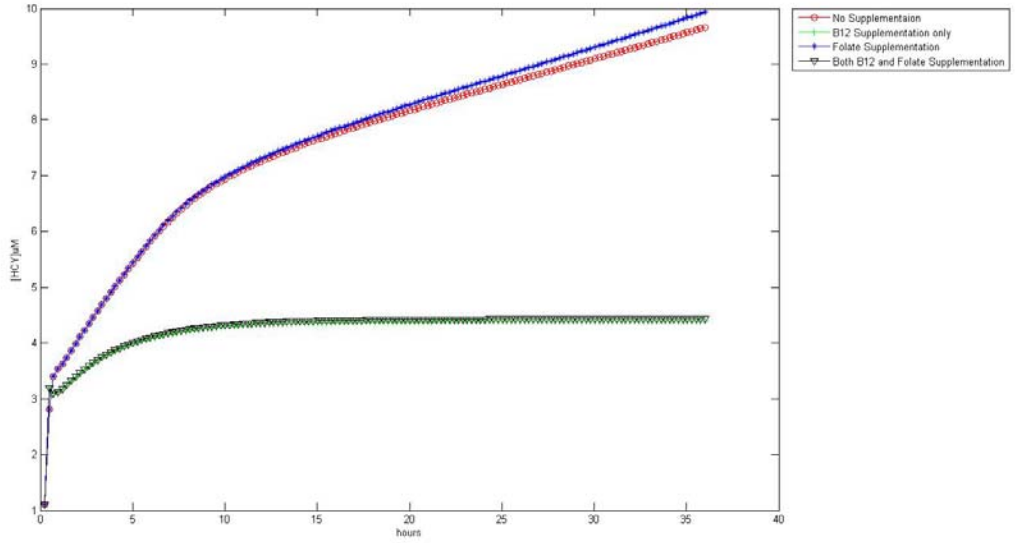

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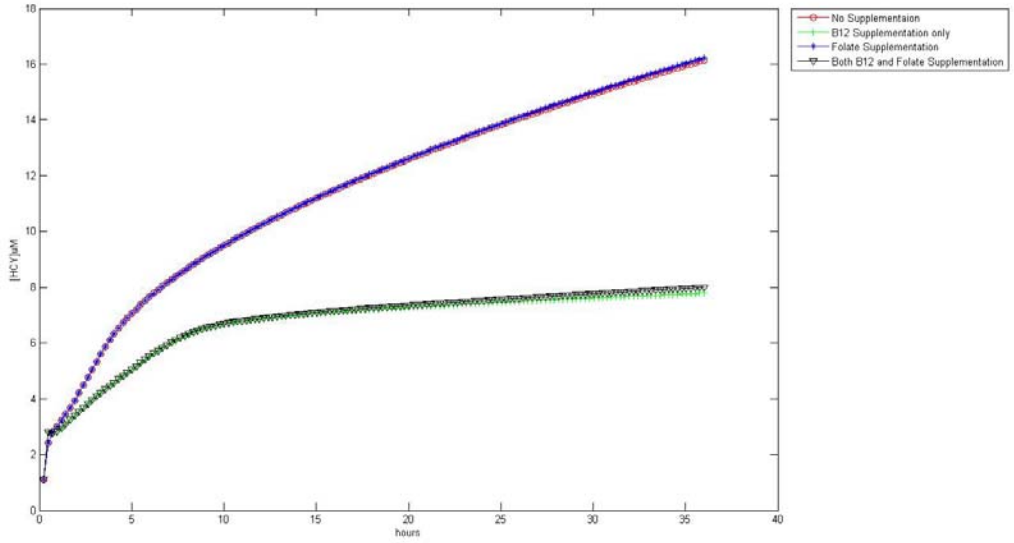

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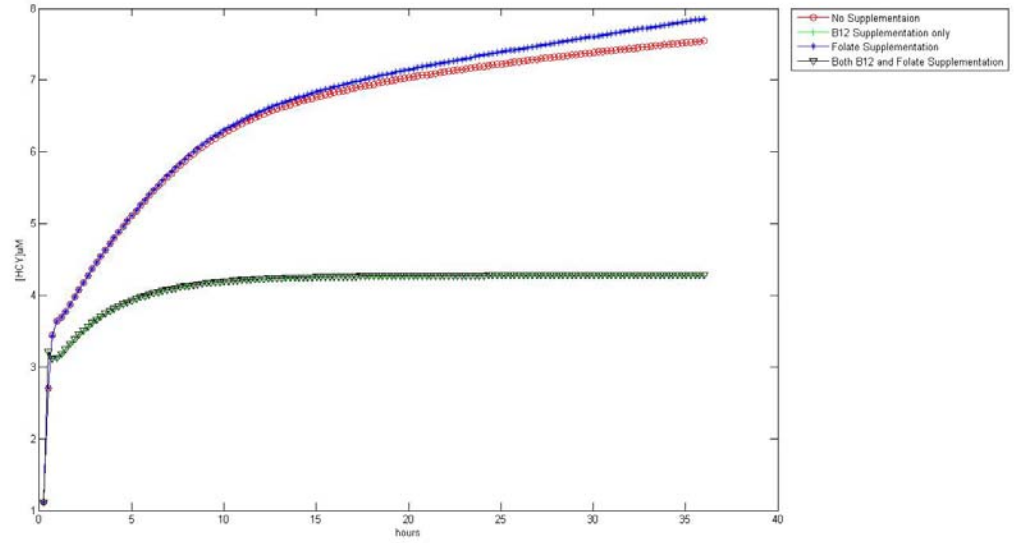

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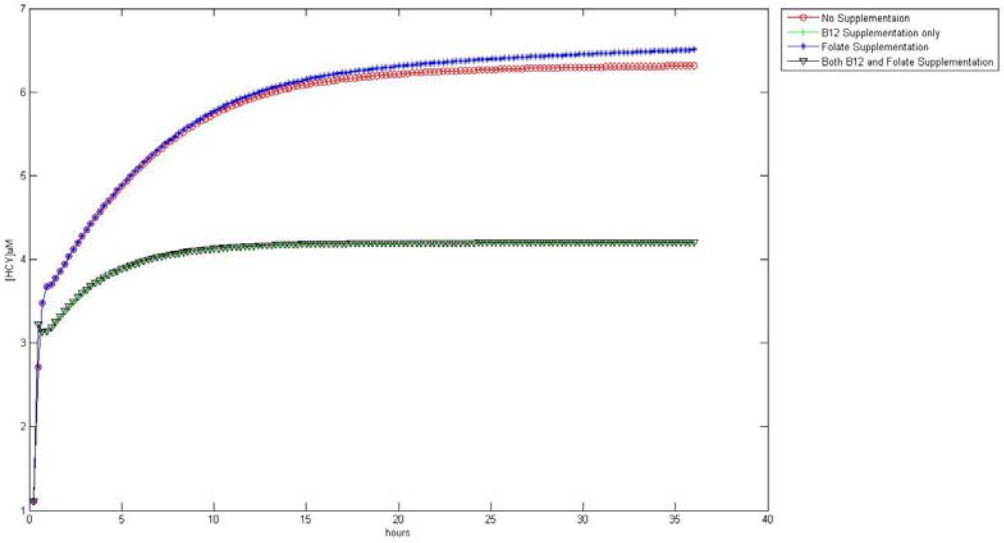

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